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Mastitis and Antibiotic Residues in Egyptian Raw Milk with Lactic Acid Bacteria Population in Dairy Products Retailed in Cairo and Giza Area

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Abstract: Raw milk and dairy products samples were collected from different locations at Cairo and Giza governorates that were microbiologically analyzed for diagnosis of mastitis and its probable bacterial causative agents, using bromothymol blue indicator test and conventional bacteriological examination. Also, a simple test for detection of antibiotic residues in the raw milk samples was used (2, 3, 5-triphenyl tetrazolium chloride). Soft cheese samples were examined for the presence and distribution of lactic acid bacteria (LAB). Samples of yoghurt, ice cream, cream and butter, were examined for the frequent distribution of different kinds of LAB and their counts using different selective media. The results revealed that all tested milk samples were negative for mastitis and antibiotics. Whereas, *Staphylococcus aureus* and *Escherichia coli* -as clinical and subclinical etiologies of mastitis were present in 45 and 57% respectively in the examined samples of raw milk. The data indicated that the highest count of total lactic acid bacteria was shown in Kariesh cheese. Meanwhile, the lower the counts of total LAB were detected in Talaga then Feta cheeses and Domiati cheese was the lowest cheese containing LAB. The results indicated that, 12.5 % of yoghurt samples were negative for LAB on different media. In ice cream, approximately 77% of the samples contained *Streptococcus* as shown on Ellikar media as mesophilic or as physcrotrophic bacteria. All cream samples showed that the average counts was ~5.0 log cycle CFU/g for *Lactobacilli* on MRS media, while the other media showed similar counts. In butter, 80% of samples showed 4.8 log cycle CFU/ g on Elliker for mesophilic LAB, while physcrotrophic LAB were in counts ~ 3.96 log cycle CFU/g.

Key words: Antibiotic · Dairy Products · Lactic Acid Bacteria · Mastitis · Pathogenic Bacteria · Raw Milk

INTRODUCTION

Mastitis is an inflammation of one or more quarters of the animals' udder usually caused by bacterial infection. Mastitis in both clinical and subclinical forms is a frustrating, costly and extremely complex disease that results in a marketed reduction in the quality and quantity of milk [1]. The most common causative pathogens of mastitis infections are *Staphylococcus aureus, Streptococcus agalactiae, S. dysgalactiae, S. uberis* and *Escherichia coli*. It can cause occasional herd outbreaks, the lesser common forms is *Pseudomonas* spp. [2].

Antibiotics infusion into the udder cure the clinical disease but may not eliminate the bacterial infection [2] and stay as residues in milk that seriously affect the public health by causing allergic reactions to residues or by the development of resistant strains of microorganisms. Furthermore, the antibiotic residues in milk might affect directly or indirectly milk industry. Different sources of antibiotic residues in milk were outlined by Radostitis *et al*. [3].

On the other hand, lactic acid bacteria (LAB) are important in the fermentation of food products (dairy, meat, vegetables, fruits and beverages), include certain species of the genera *Lactobacillus, Lactococcus, Streptococcus, Leuconostoc* and *Pediococcus* [4]. However, the presence of LAB in the local milk products such as cheese, yoghurt, ice cream, cream and butter [5], might reflects the quality and safety of milk and associated products that were the target in this study.

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The objective of this study was to determine normality or abnormality of raw milk, subsequent detection of subclinical mastitis and its causative agents and the presence of antibiotic residues in raw milk retailed in Cairo and Giza area. Also, Lactic acid bacteria population were in target for evaluation using different media, for different types of LAB bacteria in white soft cheeses, yoghurt, cream, ice cream and butter obtained from the same raw milk distribution area.

MATERIALS AND METHODS

Materials

Samples Collection: Seventy five raw milk samples, 70 samples of the Egyptian style, varieties, of white soft cheese and 80 samples of different dairy products (ice cream, yoghurt, cream and butter) were collected from different markets in Cairo and Giza governorates (from July to November, 2012).

Methods:

- Bromothymol blue (BTB) test was used for the detection of mastitis in raw milk samples [6].
- The detection of antibiotic residues in milk was carried out according to Harrigan [7] and Mhone *et al*. [8].
- The prevalence of *S. aureus* and *E. coli* in raw milk was performed according to APHA [9] and FDA [10].
- Lactic acid bacteria (LAB) in some dairy products was estimated using Elliker agar, the plates were incubated at 35°C for 2 to5 days according to Elliker *et al*. [11] and purified according to Kandler and Weiss [12].
- Lactobacilli bacteria rods were enumerated on MRS (Oxoid) agar at 35°C according to De Man *et al*. [13]. The plats were incubated at 35°C for 2 to5 days, at anaerobic condition. Also, Total lactococci were enumerated on M17 (Oxoid) agar and the plates were incubated at 35°C for 48 h according to Zambou *et al*.[14].
- *Lactobacillus bulgaricus* and *S. thermophilus*: The media of Rogosa at pH 5.5 and *S. thermophilus* agar were used for counting *L. bulgaricus* and *S. thermophilus*, respectively and the plates were incubated at 37 ºC for 48 h according to Dave and Shah [15]. The selected colonies of LAB were purified and identified according to Kandler and Weiss [12].

RESULTS AND DISCUSSION

Detection of Sub-Clinical Mastitis: One commonly used screening test for the detecting of sub-clinical mastitis is BTB test that is simple and fast test to evaluate milk status.

Results in Table (2) show that the entire raw milk samples were negative for BTB test. This means that there is no sub-clinical mastitis cases were diagnosed in all tested raw milk samples. From the foregoing results, it was observed that the BTB test has a number of practical advantages cow side use. It is simpler to perform (no mixing of reagents) and is more objective, being based on color reactions and not viscosity changes.

In Egypt, mastitis was detected in 34.12 and 9.64% of quarter milk samples collected from cases of clinical mastitis in cows and buffalo, respectively [16].

Table 1: Key locations, symbols, used for collecting raw milk and milk product samples retailed in Cairo and Giza areas.

Market/symbol	No. of samples	Location
North Giza-A	10 raw milk, 3Domiati, 2 Talaga, 1 Feta, 2 Kariesh, 2 of each of milk products*	Embaba andEl Warak
East Giza-B	5raw milk, 3Doiati, 1 Talaga, 1 Feta,1 Kariesh, 2 of each of milk products*	Elmohandseen andAgoza
West Giza-C	10 raw milk, 3Domiati, 2 Talaga, 1 Feta, 2 Kariesh, 2 of each of milk products*	El Saf andAiat
South Giza-D	10 milk, 3Domiati, 2 Talaga, 1 Feta, 2 Kariesh, 2 of each of milk products*	Haram,Sakiat meky,Omrnia
North Cairo-E	5 milk, 3Domiati, 1Talaga, 1 Feta, 1 Kariesh, 2 of each of milk products*	Shobra Khima
East Cairo-F	5 milk, 3Domiati, 2 Talaga, 1 Feta, 2 Kariesh, 2 of each of milk products*	Nasr city,Masr Gedida
South Cairo-G	5 milk, 3Domiati, 1 Talaga, 1 Feta, 1 Kariesh, 2 of each of milk products*	El Madii and Basateen
West Cairo-H	10 milk, 3Domiati, 2 Talaga, 1 Feta, 2 Kariesh, 2 of each of milk products*	Abbaseia
Hellwan-I	5 milk, 3Domiati, 1 Talaga, 1 Feta, 1 Kariesh, 2 of each of milk products*	Helowan
West Cairo-J	10 milk, 3Domiati, 2 Talaga, 1 Feta, 2 Kariesh, 2 of each of milk products*	Ain Shams
Total	75 raw milk, 70 cheese, 80 milk products*	10

*Milk products, ice cream, yoghurt, cream and butter.

Table 2: Clinical mastitis, antibiotic residues and *Staphylococcus aureus* sub clinical mastitis in raw milk samples retailed in Cairo and Giza area

		S. aureus (log_{10} CFU/mL)				Mastitis	*Antibiotic residues*
Area of samples	N0 of samples	min	max	Average	Positive samples	%	%
Giza A	10	2.6	5.34	4.5	5	0	0
Giza B	5	1.4	4.64	2.38	2	0	0
Giza C	10	1.4	5.81	5.3	6	0	0
Giza D	10	3.3	5.72	4.17	3	0	0
Cairo E	5	2.7	5.54	4.3	3	0	0
Cairo F	5	2.3	5.17	3.47	3	0	0
Cairo G	5	Nil	Nil	Nil	0	0	0
Cairo H	10	2.6	5.53	4.5	7	0	0
Hellwan I	5	2.7	5.81	4.3	3	0	0
Cairo J	10	4.4	5.54	5. 4	2	0	0
Total	75	1.4	5.3	3.30	34	0	0

Clinical mastitis and antibiotic residues, 0%, *S. aureus* sub clinical mastitis 45%

Table 3. *Escherichia coli* sub clinical mastitis in raw milk samples retailed in Cairo andGiza area

		E. coli counts (log_{10} CFU/mL)			
Area of samples	No. of samples	min	max	average	Positive samples
Giza A	10	4.17	6.41	3.48	5
Giza B	5	1.69	4.32	2.00	1
Giza C	10	2.3	6.49	3.30	6
Giza D	10	4.49	6.38	3.48	5
Cairo E	5	4.61	6.54	5.40	3
Cairo F	5	1.47	5.07	2.70	3
Cairo G	5	Nil	Nil	Nil	0
Cairo H	10	4.36	3.95	2.70	5
HellwanI	5	Nil	Nil	Nil	0
Cairo J	10	4.73	6.5	3.00	5
Total	75	1.47	6.54	3.30	33

Clinical mastitis and antibiotic residues, 0%, *E. coli* sub clinical mastitis 44%

Meanwhile, Abdelhameed and Sharaf [17] inspected 982 animals, cows and buffaloes at Kaluobia and Menofia governorates, for clinical and subclinical mastitis by CMT, PCR and conventional bacteriological tests. They found that overall prevalence of clinical and subclinical mastitis in cow's farms was 9.56 and 27.61%, respectively and in buffalo's farms was 6.00% and 17.50%, respectively. In this respect it could be remember that all dairy herds have cows with sub-clinical mastitis and the prevalence of infected cows varies from 15-75 % and quarters from 5-40 %.

Detection of Antibiotic Residues in Milk: Results of the presence of antibiotic residues in raw milk samples are shown also in Table (2). All of the raw milk samples were negative for the presence of antibiotic residues. With the *S. thermophilus* culture the characteristic pink color was not produced in all milk samples. It could be mentioned that the use of 2, 3, 5-triphenyl tetrazolium chloride can be helpful in testing antibiotic residues in milk in conjunction with organisms that are sensitive to antibiotics like *S. thermophilus*. As the test has been outlined, a person familiar with simple laboratory techniques should have no difficulty in using it to detect starter inhibitory substances in milk [18]. However, in this respect Elewa and El Fakharany [19] studied the induction of some lactic acid bacteria to certain antibiotics. They revealed that *Lactobacillus acidophilus, L. casei and S. thermophilus* were susceptible to cefadroxil monohydrate, whereas *L. rhamnosus, L. reuteri* were resistant to Erythromycin ethylsuccinate as well as, *L. casei para casei and L. reuteri* to Amoxicillin.

The Causative Bacterial Agents of Subclinical Mastitis in Raw Milk Samples

Staphylococcus Aureus Subclinical Mastitis in Raw Milk Samples: Results of detection and enumeration of *S.aureus,* as causative agent of subclinical mastitis, in 75 of raw milk samples are presented in Table (2).

The frequency of *S. aureus* by the conventional methods was 45.33 % of the samples indicating the probable of subclinical mastitis in the produced animals with the subsequent probable problems that might come up from their milks. *S. aureus* infection can occur during milking when organisms penetrate a teat canal [20].

Slightly higher counts of *S. aureus* in raw milks were obtained by Aboou Dawood [21] than that found in the current study and that obtained by Girgis *et al*. [22]. However, *Staphylococcus aureus* was the predominant mastitis pathogen (42.86%) of clinical and subclinical mastitis in cows and buffalos as reported by Abd elhameed and Sharaf [19]. In general, the obtained results revealed that > 40% of the samples exceed the count 100 CFU/mL of *S. aureus* which does not meet the Egyptian standard for raw milk.

***Escherichia coli* Sub Clinical Mastitis in Raw Milk Samples:** The suspected sub clinical mastitis causative agent *E. coli* prevalence and counts in raw milk samples using the conventional methods are shown in Table (3). Results revealed that *E. coli* counts ranged from 30 to10^4 CFU/mL, with an average of 10^3 CFU/mL in 44% of the samples by the conventional method. Also, the prevalence of *E. coli* in the tested samples, as high as obtained by Sadek, *et al*. [23], which reflected the poor hygienic conditions of production and handling of the marketed milk in Cairo and Giza governorates. Furthermore, the obtained results showed lower counts and prevalence for *E. coli* than was found by Aboou Dawood [21], but higher than that reported by Girgis *et al*. [22] and Abdel Fattah *et al*. [24]. In this respect, Hammed *et al*. [25] recorded that environmental sub clinical mastitis was diagnosed where > 2000 CFU/mL of *E. coli* and other coliforms were presented. These variations may be due to, health of animals producing, seasonal variation, the difference locations and/or the hygienic conditions of the marketed milk. Also, the hazardous consequences of making cross contamination whither indoors or outdoors might be threaten the public health with a big diverse of pathogenic types and strains of *E. coli* that might came from unhealthy animals. Awed *et al* [26] concluded that both buffaloes' and cows' milk are often of poor bacteriological quality owing to the objectionable condition under which it was produced and marketed.

Lactic Acid Bacteria in Association with Some Dairy Products: It is clearly noted that total *Lactobacilli* were enumerated on MRS, total *Lactococci* were enumerated on M17, while Rogosa at pH 5.5 was used for *L. bulgaricus* bacteria and *Streptococcus thermophilus* (ST) agar for *S. thermophilus*.

Lactic Acid Bacteria in Association with Egyptian White Soft Cheese: The distribution of the frequency collected white soft cheese samples were screened in Figure (1). The figure reveal that 43% Domiati cheese, 22% Talaga cheese, 14% Feta cheese and 21% Kariesh of the total 70 samples of white soft cheeses that were tested for LAB population and identification. Results of the percentages of LAB in different Egyptian white soft cheese samples were shown in Figure (2).The figure reveal that19% Domiati cheese, 31 % Talaga cheese 12% feta and 38 % Kariesh contained Lactic acid bacteria with different populations.

The averages of log counts of LAB on Elliker, for total LAB, M17, for *Lactococci* and MRS, for *Lactobacilli*, media are viewed in Figure (3). The data indicate that the highest count of lactic acid bacteria was shown in Kariesh cheese as 7 log cycle's CFU/g on Elliker medium. Total *Lactococci* were counted in an average count of 5 log cycles CFU/g on M17 agar. This might be due to the fermentation nature or process required for this kind of cheese making. Talaga and Fate cheese varieties turned up the next for containing LAB. They contained approximately 6.4 and 5.4 log cycle CFU/g on Elliker medium and 5.6 and 5.0 log cycles CFU/g on M17 medium, respectively. But Domiati cheese gave the counts ranged 5 log cycles CFU/g and 4 log cycles CFU/g for total LAB count and *Lactococcus* count on M17, respectively. In addition the entire of the tested samples were free from detected *Lactobacillus* spp. on MRS medium.

The obtained results indicated that lactic acid bacteria were appeared as the mainly group of nature flora for all cheese kinds and this in agreement with Tzanetakis and Litopoulou [27] results, who found that mean log counts of lactic acid bacteria ranged from 4.59 in curd to 7.95 CFU/g in Feta cheese. On the other hand, Pesic-Mikuleci and Jovanovic [28] pointing to the presence of heterofermentative *lactobacili* in soft fresh cheeses was very low. And this species were mainly isolated during the first stage of ripening and then disappeared because of their sensitivity to the environmental conditions. The cementation of this data was referred to present and distributed of lactic acid bacteria in cheese depended on: kind of milk (raw or heated), added starter or natural fermentation (Kariesh cheese); steps of manufacture, (salting, pickled); additives such as (antibacterial, spices and color) and finally the condition of environment around of the cheese.

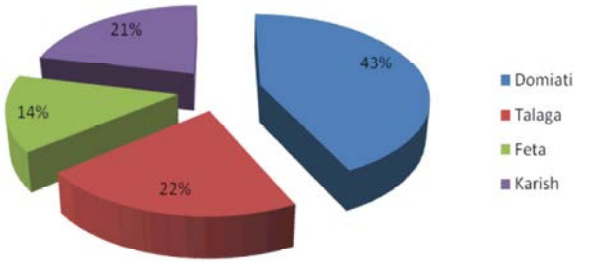

Fig. 1: Distribution of White Cheese Samples

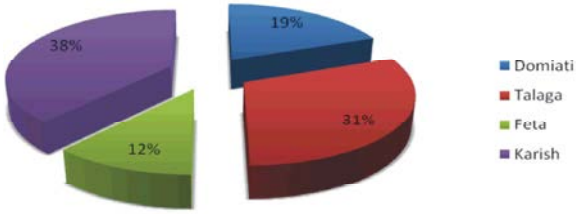

Fig. 2: Distribution of cheese samples containing lactic acid bacteria

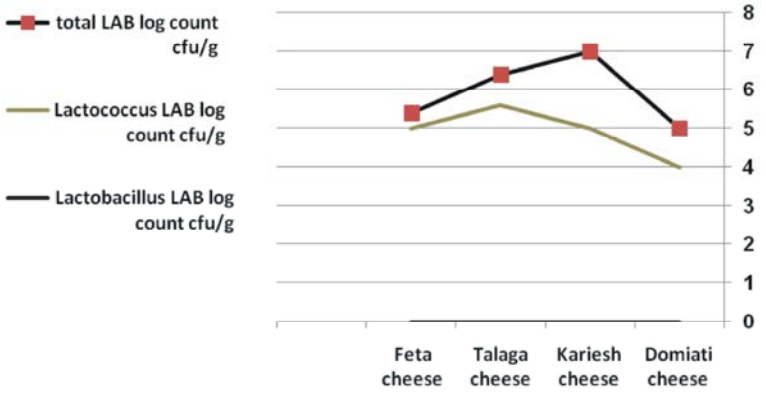

Fig. 3: Average counts of lactic acid bacteria in Egyptian white soft cheeses vended in Cairo.

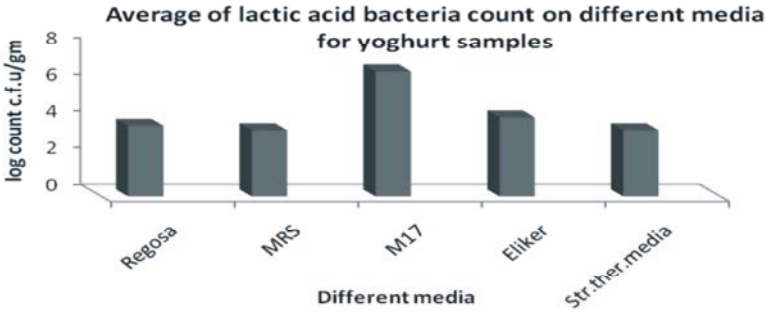

Fig. 4: Average of lactic acid bacteria in yoghurt samples on different media

Lactic Acid Bacteria in Other Different Dairy Products:

Yoghurt Samples: An average of log count for LAB at different evaluated medium was viewed in Fig (4). The data indicated that the highest count appeared on M17 media ˜8.6 log cycle. But on MRS, Rogosa and *S. thermophilus* media, the count was similar ˜ 3.7 log cycle

The results were screened the existence of LAB bacteria in yoghurt was not as yoghurt starter (*S. thermophilus* and *L. bulgaricus*) but there are different species for cocci strains.

Ice Cream Samples: The data revealed by Fig. (5) maintained to the average of count on different media as indicator to kinds strains of LAB. Mesophilic and physcotrophic counts on Elliker were approximated ˜2.9 log cycle CFU/g. The count on M17 was 3.6 log cycle CFU/g. But the less count was appeared on MRS media ˜2.3 log cycle CFU/gm. These data indicated that, most strains of LAB were cocci such as *Streptococcus*, *Lactococcus* and Enterococcus could be related to physcotrophic bacteria

Cream: The average counts on different media were shown in Fig (6) which was clarified that highest counts could be detected on MRS ~5.1 log cycle CFU/g. While others were similar in counts ~4.4 log cycle CFU/g. These data indicate that most samples contained Leuconostoc strain which counted on MRS media.But counts on other media possibility as mesosphilic Lactococcus.

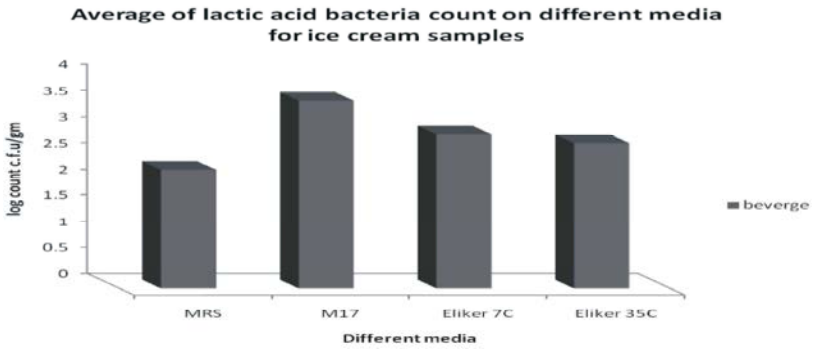

Fig. 5: Average of lactic acid bacteria in Ice cream samples on different media

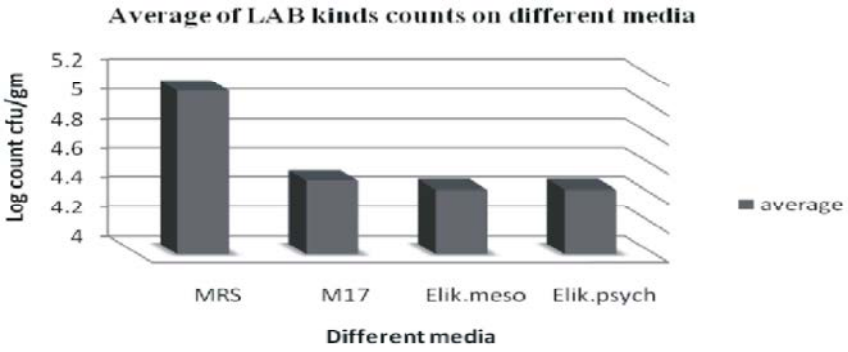

Fig. 6: Average of lactic acid bacteria in cream sample on different media

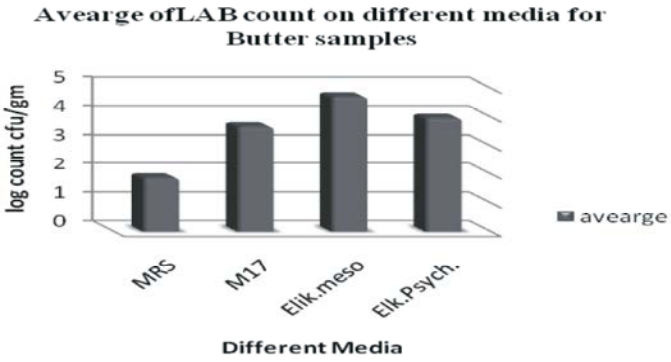

Fig. 7: Average of lactic acid bacteria in butter samples on different media.

Butter: The average range of LAB counts on different media was screening in Fig. (7). The highest count was observed on Elliker media as mesophilic ~4.7 log cycle CFU/g. But on MRS the range of viable cells was ~1.9log cycle CFU/g. The cells population counts range at M17 and physcotrophic were a like 3.66 and 3.96 log cycle CFU/g respectively.

A wide variety of LAB from traditional Egyptian dairy products that showed potentially important properties is not only valuable for practical application but they may also provide an expanded gene pool designing genetic modified strains with improved traits Aiad and Shokery [29].

CONCLUSIONS

The findings of the present study approved that examined raw milk samples were negative for clinical mastitis and no antibiotic residues could be detected. Although *S.aureus* and *E.coli* as a causative agent of mastitis could be detected in 75 and 44 % of examined raw milk samples. Therefore, hygienic milking practice and hygienic practice in the environmental should be followed. On the other hand, present of lactic acid bacteria in dairy products depended on kind of milk (raw or heated), added starter or natural fermentation, step of manufacture, additives and finally the condition of environmental around the products.

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