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Original Article

Microbiological Assessment of some Parameters of Kariesh Cheese Sold by Supermarkets and Street Vendors in Alexandria, Egypt

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Abstract

Background: Kariesh cheese is the most popular soft cheese consumed in Egypt especially in the countryside**.** Some of Kariesh cheese is produced in equipped factories, but most in farmers' homes and unlicensed places not under standard requirements for hygienic food production**.** Cheese could be contaminated by different types of microorganisms during its production, handling, distribution and storage under unhygienic conditions. Contamination with different microorganisms causes cheese spoilage and/or foodborne illnesses**.**

Objective(s): To assess some microbiological parameters of Kariesh cheese, as recommended by the Egyptian standards for Kariesh cheese No.1008/2000. A comparison of Kariesh cheese samples collected from supermarkets and street vendors was carried out.

Methods: A total of 270 Kariesh cheese samples were collected in the period between September 2015 and January 2016 from 3 randomly selected Alexandrian districts. Half of the samples (135) were collected from street vendors and the other half was collected from supermarkets. The microbiological tests performed were: total plate count, estimation of total and fecal coliforms, and detection of *E. coli*, *S. aureus* as well as yeasts and moulds.

Results: According to the Egyptian standard No.1008/2000 for Kariesh cheese parameters, only 6% and 7% of the examined Kariesh samples were satisfactory for yeasts and moulds and total plate count respectively. As regards total coliforms and *E.coli*, 44% and 48% respectively of the samples were satisfactory, while 39% of the samples were satisfactory for fecal coliforms, and around 90% were for *S. aureus.* The mean microbial counts in all tested parameters were higher in Kariesh cheese samples sold by street vendors rather than supermarkets, and this was statistically significant**.**

Conclusions: The microbiological parameters of Kariesh cheese in this study showed unacceptable high levels especially among street vendors' samples.

Keywords: Kariesh cheese, street vendors, total coliforms, fecal coliforms, *E.coli*, *S. aureus*, fungi

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INTRODUCTION

Fresh milk in Egypt is mostly used to prepare traditional products such as cheese, yoghurt, rayeb, cream, butter and ice-cream. Kariesh cheese is one of the commonest cheeses consumed in Egypt, due to its relatively low price and high nutritive value. It is a low-salt soft cheese, which is specifically popular in the countryside.(1) The manufacturing, collection and transportation conditions of Kariesh cheese vary according to the manufacturer, as urban markets differ from larger ones. Kariesh cheese is either made from pasteurized milk in factories or, from raw milk without prior heat treatment by unlicensed manufacturers.(2) Kariesh cheese, like other dairy products, has all conditions necessary for the multiplication of bacteria and fungi. The microbial load of Kariesh cheese is determined by several factors, including the quality of raw milk, heat treatment, and transportation temperature and storage conditions. Moreover, poor personal hygiene of Kariesh handlers and the presence of flies and insects are potential sources of contamination.(1) Factors that may hinder the multiplication of microorganisms in milk and cheese are refrigeration, sterilization, the addition of salts, lowering the pH and decreasing water content. The commonest method of milk sterilization is pasteurization, which destroys all the pathogens in milk, except for spore forming pathogens such as certain types of *Bacillus* spp. Other methods of sterilization such as the Ultra Heat Treatment (UHT) may be used as well to overcome the spore-forming bacteria, though this method is not commonly used. [3]

The most widespread microorganisms found in raw milk which are considered to cause milk-borne and cheese-borne diseases, include Gram negative bacilli, such as *Pseudomonas* spp., Salmonella Typhi/Typhimurim, *Escherichia coli* (*E.coli*), *Shigella*, *Klebsiella*, *Yersinia enterocolitica* and *Campylobacter jejuni*. Gram positive bacteria found in raw milk include *Staphylococcus aureus* (*S.aureus*), *Bacillus cereus*, *Micrococcus*, *Aerococcus*, Clostridia spp., *Listeria monocytogenes* and *Lactococcus*.[3,4]

The microbiota of freshly pasteurized milk consists primarily of thermoduric bacteria and spores that were present in raw milk before pasteurization. When milk/cheese is refrigerated, Gram-negative psychrotrophic species may gain entry as post-pasteurization contaminants. These bacteria grow and cause spoilage of cheese and/or gastroentertitis manifested as fever, nausea, vomiting, diarrhea, general malaise and weakness. Consumption of large amounts of contaminated cheese may result in outbreaks. [3,5]

The Egyptian standards for Kariesh cheese No.1008/2000 indicate (among other parameters) that the total coliforms should be less than 10 CFU/g; *E. coli* absent in 1 g, *S. aureus* (coagulase- positive) absent in 1 g, yeasts and moulds less than 400CFU/g. [6]

This study aimed at assessing the above-mentioned microbiological parameters of Kariesh cheese, and estimating the percentage of acceptable Kariesh cheese in Alexandria market. A comparison was made between Kariesh cheese sold at some supermarkets and street vendors in Alexandria.

METHODS

In the current study 270 Kariesh cheese samples were collected in the period between September 2015 and January 2016. Half of the samples (135) were collected from street vendors and the other half was collected from supermarkets. Samples were also collected to cover 3 randomly selected districts in Alexandria, so that 90 samples were collected from West, Mid, and East districts of Alexandria. Each Kariesh cheese sample was accompanied by a sheet including the date, site, time of collection/temperature and name of manufacturer (brand or farm name). All samples were transported in an icebox to the laboratory with minimal delay.

Twenty five grams of the Kariesh cheese were mixed with 225ml of sterile peptone water in a sterile stomacher plastic bag and were blended thoroughly in the stomacher for 1-2 minutes for complete homogenization. This constituted the 1:10 dilution of the original sample.

Bacteriological Examination

All the microbiological procedures were performed according to the Bacteriological Analytical Manual (BAM, 2013). [7] All Kariesh cheese samples were subjected to the following tests:

I. Total plate count: [7]

Ten fold dilutions were prepared from the sample homogenate in diluent buffer to obtain 10^{-2}, 10^{-3}, 10^{-4} dilutions. One ml of each dilution was pipetted into duplicates of sterile Petri dishes. Twenty ml of melted sterile plate count agar were then poured into the Petri dishes and the contents were mixed thoroughly by rotating the plate several times. When the media has solidified, the plates were inverted and incubated at 37°C for 48 hours. Plates having 30-300 colonies were counted using a Quebec counter, and the average number/plate was multiplied by the dilution factor to obtain the total colony forming units (CFU)/gm.

II. Total coliforms, fecal coliforms and *E.coli* [7]

Multiple tube dilution method was performed for the total and fecal coliforms. Presumptive test for total coliforms was done using lauryl sulfate tryptose broth (LST) inoculated with 1 ml of previously prepared 1:10, 1:100 and 1:1000 dilutions. Tubes containing inverted Durham tubes were used for gas detection, and then incubated for 24 -48 hours at 37°C. All LST tubes showing both turbidity and gas within 48 hours were recorded and the Most Probable Number (MPN) was obtained from MPN food tables for the recorded 3 tube dilutions. Results were recorded as the presumptive MPN of coliform bacteria per gm.

A confirmed test was done on all presumptive positive tubes showing gas. These were subcultured into tubes of Brilliant Green Lactose Bile Broth (BGLBB) and EC broth. All BGLBB tubes showing gas were incubated at 35-37°C for detection of total coliforms (TC) for 24-48 hours, while tubes of EC broth were incubated at 44.5 ± 0.2°C for 24 hours in a circulating covered water bath for detection of fecal coliforms (FC). Positive results were tubes showing turbidity and gas. The MPN was calculated from McCrady's probability tables.

Finally, a completed test for *E.coli* was performed. Positive EC tubes from the previous confirmed test were streaked on eosin methylene blue (EMB) plates and incubated for 24 hours at 37° C. Typical nucleated (dark centered) colonies were subjected to the following tests:- Gram stain, Triple sugar iron (TSI) and Indole, methyl red, Voges-Proskauer and Simmmon's Citrate (IMViC). *E.coli* were identified as Gram negative short non- spore forming rods. Typical TSI result for *E.coli* was an acid slant/acid butt with gas, but without H_2S. IMViC results for *E.coli* were positive indole and MR, but negative VP and citrate tests.

III. *Staphylococcos aureus*[7]

One ml of each of the prepared dilutions was plated onto two plates of Baird-Parker agar with added egg-yolk tellurite enrichment. Inverted plates were incubated for 48 hours at 37°C. One or two suspected colonies were picked and Gram stained. Typical *S. aureus* colonies were Gram positive cocci in clusters. Tube coagulase was performed to confirm *S. aureus* (coagulase positive). Briefly, rabbit plasma was inoculated with a staphylococcal colony (i.e., Gram-positive cocci which are catalase positive) and the tube was then incubated at 37 °C for 3 hours. If negative, then incubation was continued up to 18 hours. Coagulase positive cultures were considered *S. aureus*.

IV- Yeasts and Moulds[7]

Ten grams taken from each Kariesh cheese sample were diluted in 90 ml of sterile solution of 2% (w/v) sodium citrate and homogenized in a stomacher. For all samples, tenfold serial dilutions were prepared and the numbers of yeasts and moulds were determined by surface plating on SDA after incubation at 25°C for 5 days. Yeast colonies were convex, smooth, creamy-white in color with regular borders. Moulds had variable morphologies (cottony, fuzzy, granular, and powdery) and colors ranging from green, white and black. The number of CFU was counted and multiplied by the dilution factor.

Statistical analysis[8]

Statistical analyses were conducted using the software: Statistical Package for the Social Sciences (SPSS) version 20. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected counts less than 5, correction for chi-square was conducted using Fisher's Exact test or Monte Carlo correction. Comparison between the studied groups was also done using independent t-test, while for abnormally distributed data; comparison between them was done using Mann Whitney (Z-test). Statistical significance was set at 5% ($P < 0.05$).

RESULTS

During this 5- month study, a total of 270 Kariesh cheese samples were studied for bacteriological parameters recommended by the Egyptian Standards for Kariesh cheese. The samples were equally collected from supermarkets and street vendors (135 samples each). The total plate count of Kariesh samples had a mean of $2.14x10^8$CFU/g and a median of $1.35x10^8$ CFU/g. *S. aureus* had a high mean of $4.19x10^8$ CFU/g however, its median count was 0.0 CFU/g. This was due to the uneven distribution of *S. aureus* counts among samples (i.e: samples had either no growth at all, or, extremely high counts of *S. aureus*). Yeasts and moulds had high mean and median counts $9.09x10^7$ and $5.70x10^7$ CFU/g, respectively (Table 1).

There was a highly statistical difference based upon the seller of Kariesh cheese as regards the medians of total plate count, total coliforms, fecal coliforms, *E. coli* and yeasts and moulds. Street vendors had significantly higher mean counts of the aforementioned parameters and were as follows: total plate count= $1.59x10^8$CFU/g, total coliforms= $2.10x10^2$MPN/g, fecal coliforms= 2.80 x10MPN/g, *E. coli*= 2.20 x10MPN/g and yeasts and moulds= 7.80x107CFU/g. Despite the high mean of S. aureus among street vendors (1.26x109 CFU/g), athough, the difference was not statistically significant (Table 2).

Table (1): Means and medians of the studied parameters of 270 Kariesh cheese samples (Alexandria, Egypt)

Parameters	Min. – Max	Mean ± SD	Median
Total plate count (CFU/g)	$8.01x10^5 – 2.28x10^9$	$2.14x108 ± 2.50x10^8$	$1.35x10^8$
Total coliforms (MPN/g)	$0.02 x10 – 1.10x10^3$	$3.68x102±4.71x10^2$	6.95 x10
Fecal coliforms (MPN/g)	$0.0 – 1.10x10^3$	$1.86x102±3.52x10^2$	2.05 x10
E. coli (MPN/g)	$0.0 – 1.10x10^3$	$4.53 x10 ± 1.60x10^2$	0.3 x10
S. aureus (CFU/g)	0.0– 1.13x1010	$4.19x108±6.88x10^9$	0.0
Yeasts and Moulds (CFU/g)	$2.40x102-9.40x10^8$	$9.09x107±1.15x10^8$	$5.70x10^7$

It appears from table (3) that most of the 135 Kariesh cheese samples purchased from street vendors were unsatisfactory according to the studied parameters, where 95.56% of those samples yielded > 400 CFU/g yeasts and moulds, 85.93% yielded ≥ 10 MPN/g total coliforms, 82.97% yielded fecal coliforms,73.33% yielded E.coli and 12.6 % yielded S. aureus. On the other hand, most of the 135 Kariesh cheese samples purchased from supermarkets were satisfactory according to the aforementioned parameters except for the yeasts and moulds, where only 6.7% were satisfactory. The samples purchased from supermarkets were significantly less contaminated by the tested parameters than those from street vendors, except for yeasts and moulds counts. In addition to the above parameters, as interpreted according to the maximum limit proposed by Ottogalli and Rondinini, and Ram et al. 9, 10, the results of the plate count of the studied Kariesh samples showed that 92.6% were unsatisfactory. There was a statistical difference between different brands of Kariesh cheese in the mean of the counts of: total plate count, total coliforms, fecal coliforms, E.coli and yeasts and moulds. The highest total plate count was found in brand (J) which had a count of 4.96 x108CFU/g, while the lowest count was found in brand (N) which had a count of 8.10 x105 CFU/g. Brand (B) had the highest total coliforms (8.48 x103MPN/g), fecal coliforms (6.10 x103MPN/g) and E. coli (3.69 x103MPN/g). On the other hand, three brands (J, N and H) showed no growth of total coliforms, fecal coliforms, E. coli or S. aureus. Therefore, their only contaminant was yeasts and moulds which constituted the whole total plate count. Brand (J) showed the highest yeasts and moulds count (1.30 x109 CFU/g), in contrast to brand (H) which had the lowest count (10.0 x105 CFU/g).

Table (2): Comparison between supermarkets and street vendors regarding the studied parameters in 270 Kariesh samples (Alexandria, Egypt)

Parameters	Seller		p value
	Supermarkets	Street vendors	
Total plate count (CFU/g)			
Min. – Max.	8.0x105 –9.5x10^{8}	2.0x106- 2.28x10^{9}	
Mean ± SD	1.80x108±2.2x10^{8}	2.50x108±2.7x10^{8}	<0.001*
Median	9.20x107	1.59x108	
Total coliforms (MPN/g)			
Min. – Max.	0.15 – 1.1x10^{3}	3.0 – 1.1x10^{3}	
Mean ± SD	1.88 x102±3.9x10^{2}	4.59 x102±4.8x10^{2}	<0.001*
Median	1.45 x10	2.10x102	
Fecal coliforms (MPN/g)			
Min. – Max.	3.0 – 1.1x10^{3}	0.11 – 1.1x10^{3}	
Mean ± SD	1.54 x102±3.6x10^{2}	2.02 x102±3.4x10^{2}	<0.001*
Median	7.40	2.80 x10	
***E.coli* (MPN/g)**			
Min. – Max.	3.0 – 1.1x10^{3}	3.0 – 2.1x10^{3}	
Mean ± SD	7.60 x10±2.4x10^{2}	1.11 x102±3.3x10^{2}	0.001*
Median	7.50	2.20 x10	
***S.aureus* (CFU/g)**			
Min. – Max.	0.0 – 2.2x10^{3}	0.0- 1.13x10^{10}	
Mean ± SD	7.80 x 102±3.2x10^{2}	1.26x109± 9.7x10^{8}	0.101
Median	0.0	0.0	
Yeasts and Moulds (CFU/g)			
Min. – Max.	2.4x102- 9.4x10^{8}	1.0x106 – 8.2x10^{8}	
Mean ± SD	7.17x107±1.1x10^{8}	1.05x108±1.1x10^{8}	<0.001*
Median	2.60x10^{7}	7.80x10^{7}	

p value for Mann Whitney test *: Statistically significant at $p \leq 0.05$

As regards Kariesh samples sold by street vendors, the means were significantly different in fecal coliforms, E. coli and yeasts and moulds. Almost half of the samples (n=77) were originally from Albehera farms. These samples had also the highest S. aureus count (3.77x109 CFU/g). The highest total plate count was found in samples from Tanta farms (3.08x108 CFU/g), followed by samples from Albehera farms (2.77x108 CFU/g). Alsyof farms showed the highest count of total coliforms, fecal coliforms and E. coli (6.95x102 MPN/g) each. They were followed by Tanta farms which had counts of total coliforms (6.76x102 MPN/g), fecal coliforms (2.96x102 MPN/g) and E. coli (7.58x103 MPN/g). Almahmodia farms showed the highest yeasts and moulds count (1.81x108 CFU/g), in contrast to Domiat farms which had the lowest count (5.59x107CFU/g) (Table 4). Regarding all the studied parameters except for S. aureus there was a significant difference between samples obtained from supermarkets and those obtained from street vendors (farms).

Table (3): Category of the studied 270 Kariesh cheese samples according to their studied parameters and seller of Kariesh samples (Alexandria, Egypt).

a.Total plate count (CFU/g)

Seller		Total plate count(CFU/g)				X^2 F.E.T.	*p*
		Satisfactory ≤105		Unsatisfactory >105			
		No.	%	No.	%		
Supermarkets	135	8	5.9	127	94.1	0.864	0.353
Street vendors	135	12	8.9	123	91.1		
Total	270	20	7.4	250	92.6		

b. Total coliforms (MPN/g)

Seller		Total coliforms (MPN/g)				X^2 F.E.T.	*P*
		Satisfactory <10		Unsatisfactory ≥10			
		No.	%	No.	%		
Supermarkets	135	100	74.07	35	25.93	98.59*	<0.001*
Street vendors	135	19	14.07	116	85.93		
Total	270	119	44.07	151	55.93		

c. Fecal coliforms (MPN/g)

Seller		Fecal coliforms (MPN/g) Satisfactory absent No.	%	Unsatisfactory Any growth No.	%	X^2 F.E.T	*P*
Supermarkets	(135)	82	60.74	53	39.26		
Street vendors	(135)	23	17.03	112	82.97	54.25*	<0.001*
Total	(270)	105	38.89	165	61.11		

d. *E.coli* (MPN/g)

Seller		*E.coli* (MPN/g) Satisfactory absent No.	%	Unsatisfactory Any growth No.	%	X^2	*P*
Supermarkets	135	93	68.89	42	31.11		
Street vendors	135	36	26.67	99	73.33	48.229*	<0.001*
Total	270	129	47.78	141	52.22		

e. *S.aureus* (CFU/g)

Seller		*S.aureus* (CFU/g) Satisfactory absent No.	%	Unsatisfactory Any growth No.	%	X^2	*P*
Supermarkets	135	128	94.8	7	5.2		
Street vendors	135	118	87.4	17	12.6	4.573*	0.032*
Total	270	246	91.1	24	8.9		

f. Yeasts and moulds (CFU/g)

Seller		Yeasts and moulds 2(CFU/g) Satisfactory ≤400 No.	%	Unsatisfactory >400 No.	%	X^2	*P*
Supermarkets	135	9	6.67	126	93.33		
Street vendors	135	6	4.44	129	95.56	0.635	0.425
Total	270	15	5.56	255	94.44		

x^2: Chi square test (F.E.T.): Fisher Exact Test *: Statistically significant at $p \leq 0.05$

Table (4): Means of the studied parameters according to the manufacturer of 270 Kariesh samples (Alexandria, Egypt)

Seller	Manufacturer	No. of samples	Total plate count (CFU/g)	Total coliforms (MPN/g)	Fecal coliforms (MPN/g)	*E.coli* (MPN/g)	*S.aureus* (CFU/g)	Yeasts& Moulds (CFU/g)
Supermarkets (n = 135)	A	5	3.16x108	1.45 x10	0.82 x10	0.42 x10	-	3.14x108
	B	15	3.53x107	8.48 x103	6.10 x103	3.69 x103	-	2.98 x107
	C	5	1.20 x108	0.82 x10	0.36 x10	0.36 x10	-	7.16 x107
	D	35	9.40 x107	0.88 x10	0.47 x10	0.52 x10	-	4.2x107
	E	5	4.05x108	4.39 x10	1.29 x10	1.06 x10	-	1.37x108
	F	5	3.02 x107	0.33 x10	0.30 x10	-	-	7.60 x107
	G	5	3.60 x107	1.55 x10	0.92 x10	0.82 x10	-	1.94 x107
	H	5	4.40 x107	-	-	-	-	10.0 x105
	I	7	1.69 x108	2.32 x10	1.04 x10	0.79 x10	1.69 x103	4.93 x107
	J	10	4.96 x108	-	-	-	-	1.30 x109
	K	5	1.36x107	0.36 x10	-	-	-	1.44 x107
	L	7	1.81x108	0.70 x10	0.48 x10	0.33 x10	2.90 x102	1.37 x107
	M	6	3.40x108	4.33 x10	1.77 x10	1.38 x10	9.50 x102	1.87 x107
	N	5	8.10 x105	-	-	-	-	6.86 x107
	O	15	3.41x108	1.76 x10	0.98 x10	6.0	-	2.08x107
p			<0.001*	0.004*	0.009*	0.008*	0.090	<0.001*
Street vendors (n = 135)	Farms (Albehera)	77	2.77x108	4.72 x102	1.71 x102	4.21 x10	3.77x109	1.06x108
	Farms (Almahmodia)	7	1.73 x108	3.15 x102	3.30 x10	0.0	1.76 x103	1.81x108
	Farms (Alsyoff)	2	1.50x108	6.95 x102	6.95 x102	6.95 x102	-	1.42x108
	Farms (Domiat)	14	2.28 x108	4.06 x102	2.32 x102	7.04 x10	3.50 x 102	5.59 x107
	Farms (Kafr Alsheekh)	22	1.64 x108	3.30 x102	1.86 x102	1.68 x102	1.39 x 103	9.61 x107
	Farms (Tanta)	13	3.08x108	6.76 x102	2.96 x102	7.58 x103	-	1.24 x108
p			0.368	0.179	0.026*	0.03*	0.38	0.022*

DISCUSSION

Dairy products can get contaminated with different microorganisms, including Gram-negative psychrotrophs, coliforms, yeasts, and moulds. In addition, various bacteria of public health concern such as *Salmonella* spp., *L. monocytogenes*, *Campylobacter jejuni*, *Y. enterocolitica*, pathogenic strains of *E. coli* and enterotoxigenic strains of *S. aureus* may also be found in milk and dairy products. This might lead them to be unfit for consumption and may constitute a public health hazard.[3,5] There are many varieties of white soft cheese depending on the technique of manufacture, salt percentage and many other factors. Kariesh cheese is one of the most popular soft cheeses in Egypt due to its low price and high nutritive value. Its manufacture in farms depends on natural fermentation of raw skimmed milk by lactic acid producing bacteria which are normally present in raw milk. Its manufacture in factories depends on pasteurized rather than raw milk. [1, 2]

The Egyptian Standards for Kariesh Cheese edict of Egyptian government No.1008/2000 [6], in addition to several researchers such as Ottagalli et al., and Ram et al.,[9,10], categorize a sample as unsatisfactory if it yielded any of the following:-

≥ 10 total coliforms MPN/g. Any growth of *E.coli*, fecal coliforms and/or *S. aureus* in 1 gm of food sample.

≥ 400 yeasts and moulds CFU/g.

> 10^5 total plate count CFU/g.

The present work was carried out to investigate some important bacteriological parameters of Kariesh cheese, which are total coliforms, fecal coliforms, *E.coli*, *S.aureus* and yeasts and moulds.

Total bacterial count is a useful indicator for the microbiological status of cheese. A high viable count often indicates contamination of raw materials, unsatisfactory sanitation, or unsuitable time and temperature during storage and/or production.[11] The Egyptian standards for Kariesh cheese mentioned no account on the acceptable level of total bacterial count, but they obligate the pasteurization or any equivalent heat treatment of the cheese milk with the addition of powerful starter culture.[6,8] The result of the current study revealed that the total bacterial count had a mean of 2.14×10^8 CFU/g (table 1). On the other hand, a study by Kaldes et al., in Minia (1997, Egypt) reported much lower total plate count in Kariesh cheese (1.1×10^4 CFU/g).[11] Nearly similar results were obtained by other studies carried out in Egyptian cities. Baraheem et al., (Alexandria, 2007) found a total plate count of 1.1×10^9 CFU/g in all Kariesh samples examined. [12] Similarly, Aman (Kafr Elsheikh, Egypt) and Moussa et al., (Menoufia, Egypt) reported total bacterial count in Kariesh cheese of 2.6×10^8 CFU/g and 3.3×10^7 CFU/g, respectively. [13, 14] Ibrahim et al., showed a higher value of total bacterial count in Kariesh cheese $7.2 \pm 0.1 \times 10^{10}$ CFU/g . [15] Extremely higher total plate counts were recorded by Amer et al., in Zagazig (Egypt), who reported a count of 1.0×10^{13} CFU/g. [16] Seifu et al., 2013 [17] observed that traditional Ethiopian soft cheese samples had a total viable bacterial count up to 6.9×10^7 CFU/g and their results were similar to those of Ashenafi 1990 [18] who reported that the majority (92%) of such cheese samples collected from open markets in Ethiopia had aerobic mesophilic counts greater than 107 CFU/g.

Some investigators such as Ottagalli and Rondinini et al., and Ram et al., proposed a maximum limit of total bacterial count for soft cheese from 10^4 - 10^5 CFU/g. [9,10] According to such standards, 92.6 % of the examined samples of the present study would be considered unsatisfactory, denoting very high contamination levels. Coliforms and fecal coliforms have probably received more attention than most other groups of bacteria from public health point of view on account of their importance as indicator organisms for predicting unhygienic conditions during production, handling and processing of food. They are responsible for spoilage of milk and its products, as well as being an indicator for possible presence of other pathogens.[3, 5] The distribution of coliforms in cheese is dependent on pasteurization, pH, water activity and milk type. Moreover, the temperature of storage and transportation of cheese affect the number and type of coliforms present, since some coliforms are psychrotolerant, others are thermotolerant, while a third group include ubiquitous coliforms that also include some thermotolerant coliforms. Masiello et al. (2016) reported that genera such as *Serratia*, *Hafnia*, *Rahnella*, *Buttiauxella*, and *Leclercia* are environmental coliforms, and that some strains of them are able to grow in milk at refrigeration temperatures.[3, 19] In the present study the means of the total and fecal coliforms count in all samples were 3.68×10^2 and 1.86×10^2 MPN/g, respectively. Accordingly, 44.07% and 38.89%, respectively of these samples would be considered satisfactory by the Egyptian standards (tables 3 b and c). The mean of total coliforms in the present study is similar to that of Baraheem et al., who reported total coliforms presence in all examined Kariesh samples (open and packed) with a mean of 7.3×10^2 MPN/g for open cheese and 1.6×10^2 MPN/g for packed ones. [12] Higher results were reported by Metwalli (2011). [20] who reported the numbers of coliforms bacteria in the examined samples with a mean of 1.50×10^4 MPN/g. Esho et al., in Japan 2013 observed that 27.3%of the examined soft cheese samples were positive for coliforms with values up to 3.0×10^6 MPN/g.[21] These findings are higher than the results of the current study. As already mentioned, the presence of coliforms bacteria indicates possible fecal contamination in food, as well as the possibility that other intestinal pathogens such as enteropathogenic *E.coli* may also be present. [3,5] The presence of coliforms in cheese and their relation to entropathogenic *E. coli* in soft cheeses have been reported in previous studies. After an outbreak of foodborne disease caused by enteropathogenic *E. coli*, the presence of these microorganisms in cheese acquired additional significance. [22]

The public health importance of *E.coli* is that it is implicated in gastrointestinal illnesses as severe cholera-like syndrome, gastroenteritis, epidemic diarrhea and cases of food poisoning. [22] Shiga toxin-producing *E. coli* was the cause of outbreaks resulting in hemorrhagic colitis and lethal hemolytic uremic syndrome. [23] It is worthily to note that presence of *E.coli* in milk and milk products is an indication of direct or indirect fecal contamination. The contamination may be through contaminated hands and/or milk in which the organisms can survive well in improperly heat-treated milk and some strains can survive pasteurization.[22, 23] In the present study, 47.78% of samples were satisfactory for *E.coli* according to the Egyptian standards (table 3 (d). Nearly similar results were obtained by El-Sayed et al., (2011). [24] Lower results were reported by Brien et al.,[25] (Ireland) who concluded that *E. coli* was absent (0.0 MPN/g) from 210 pasteurized milk cheeses tested.

S.aureus was detected in Kariesh cheese samples with a mean of $7.06x10^8$ CFU/g, and accordingly, 91% of these samples were considered satisfactory by the Egyptian standards (table 3). These results were slightly close to those reported by Ibrahim et al., 2015 [15] (1.2×102 CFU /g). Higher results were obtained by El-Sayed et al.,(2011)[24] and Metwalli (2011) [20] who found that the *S. aureus* was detected in all their Kariesh cheese samples with an average of $2x10^5$ CFU/g. Nunes et al., (2013) cited that *S. aureus* outbreaks occurred in Brazil and were linked to soft cheese consumption.[26] Nearly similar results obtained by Al-tahiri (2005)[27] who revealed the presence of *S.aureus* in all traditional cheeses with mean values of 5x10*3* CFU/ml. Tondo et al., [28] reported that 35.2% of food handlers were asymptomatic carriers of *S. aureus*, and that 90.4% of raw milk samples contained *S. aureus*.

The presence of *S.aureus* in cheese usually indicates contamination of milk from diseased udder or external surface of the dairy animals, or from contaminated, unclean hands of the dairy -workers or from their sneezing and coughing. *S. aureus* may be the main cause of several food intoxication outbreaks for their production of heat stable enterotoxins, which can cause food poisoning with levels as low as 0.5ng-g-1.[29] It is widely recognized that fungi are an important component of the micro flora of many cheese varieties. The high prevalence of fungi in cheese is due to several factors which are: the ability to ferment/assimilate lactose, produce extracellular lipolytic and proteolytic enzymes, utilize lactic and citric acid, grow at 10°C and their relative resistance to cleaning compounds and sanitizers. [30] Despite that Soliman and Aly.[30] recorded that yeasts cause discoloration and changes in the texture of cheese, nothing was observed regarding the physical appearance of samples in this present study. Yeasts and moulds were detected in all tested samples, with a mean of $9.09 x10^7$ CFU/g (table 2).

According to the Egyptian standards, only 6% of Kariesh samples were satisfactory (table 3- f). Nearly similar findings were reported by El-Sayed et al. [24] On the other hand, Soliman and Aly, [30] observed a lower total yeasts and moulds count in Kariesh cheese samples with a mean of $7.89 x 10^1$ CFU/g. Concerning the difference between results of Kariesh samples sold at supermarkets and those sold by street vendors (tables 2 and 3), it was found that those sold by street vendors had significantly higher counts of total coliforms, fecal coliforms, *E.coli* and yeasts and moulds as compared to samples collected from supermarkets. This might be due to high microbial load in raw milk, difference in starter culture, contaminated utensils and environment, inadequate transportation temperature, storage and handling. In addition, flies and insects can transmit bacteria to cheese, especially when cheese is sold uncovered and without containers. Street vendors use their bare hands to handle Kariesh cheese, and this is an additional source of contamination. Low hygienic practices adopted by street vendors and the lack of governmental supervision on foods sold on the streets are responsible for such high microbial contamination. Several studies have also reported similar results, where cheese made from raw milk yielded a higher microbial load than those made from pasteurized milk. Trmcic et al.,[31] (USA, 2016) found that raw milk cheese was 4.6 –times more likely to be positive for coliforms than pasteurized milk cheese (42% and 21%, respectively). Since pasteurization destroys the thermolabile coliforms, the presence of coliforms in cheese in this case would signify post-pasteurization contamination.[31]

In the present study, 15 brands of Kariesh cheese were collected from different supermarkets. Those brands were significantly different as regards counts of total plate count, total coliforms, fecal coliforms, *E.coli* and yeasts and moulds (table 4). As already mentioned, the most prevalent organisms isolated from all brands of Kariesh cheese were yeasts and moulds. They were isolated from all different brands. Both the mean and median of yeasts and moulds were the highest among all other parameters. The least commonly isolated organism was *S.aureus*, which had a median of 0.0 CFU/g. Brands (H, J and N) were comprised solely of yeasts and moulds, while brand (B) had high levels of total coliforms, fecal coliforms and *E.coli* as well. This difference in microbial composition between brands might be attributed to differences in temperature, salt composition, pH, water activity. Low salt, pH> 5.0, water activity > 0.93 and high storage temperature favour the growth of coliforms, while low pH, low moisture content, high salt concentration and refrigeration of these products favour fungal growth.[32, 33]

In the present study, samples from 6 different farms were studied. Fecal coliforms, *E.coli* and yeasts and moulds were significantly different between various farms which may be due to difference in the duration of transportation from the farms to street vendors. Moreover, difference in production technique, concentration of starter culture and different cleaning procedures all contribute to variability in the microbial load of Kariesh cheese.[27,32] Samples from farms in Alsyoff had no growth of *S.aureus*, while those samples from Almahmodia farms had the lowest counts of fecal coliforms and *E.coli*. This might be due to the relatively closer distance of these farms to Alexandria.

CONCLUSION

To sum up, according to the Egyptian standards, No.1008/2000 [6], the results of the present study showed the following:-

- Only 6-7% the Kariesh samples were satisfactory for yeasts and moulds and total plate count respectively.
- Almost half of the samples were satisfactory for total coliforms and *E.coli*.
- Almost one third of the samples were satisfactory for fecal coliforms.
- Almost 90% of the Kariesh samples were satisfactory for *S. aureus*.

The microbiological quality of Kariesh cheese in this study indicates insufficient sanitation during manufacture and handling this type of cheese. Kariesh cheese is sold uncovered and without containers where the risk of contamination is high, so it is considered as a good medium for the growth of different types of spoilage and pathogenic microorganisms. The implementation of ‘‘Good Manufacturing Practices’’ in the production of traditional cheese is fundamental for preventing contamination.

RECOMMENDATIONS

- Better governmental supervision on supermarkets selling kariesh cheese with regular sampling of their products.
- The parameters of the Egyptian standards should be fulfilled in the samples of kariesh cheese; otherwise the cheese should be discarded.
- Supermarkets are recommended to sell only brands of kariesh cheese that are known to have adequate microbiological parameters. Supermarkets are also recommended to maintain a suitable temperature for transportation and storage of kariesh cheese.
- It is not recommended to buy kariesh cheese from street vendors due to lack of governmental supervision on their products.

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