Archives of Razi Institute, Vol. 77, No. 6 (2022) 2319-2328

DOI: 10.22092/ARI.2022.358618.2268

Original Article

Evaluation of the Bacterial Load in the Raw Dairy Products in Baghdad, Iraq

Abed Rabba Al-Shuwaili, M[1 *], Saab Khudhir, Z[2]

1. Department of Public Health, College of Veterinary Medicine, University of Kufa, Kufa, Iraq
2. Department of Public Health, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Received 7 May 2022; Accepted 8 June 2022

Corresponding Author: mahdia.dhadir@uokufa.edu.iq

Abstract

Milk and cheese were recognized as highly nutritious food due to their high protein, fat and minerals (calcium, phosphorus, iron, and vitamins). Accidental contamination of food through the handlers could have resulted in many kinds of bacteria especially Staphylococcus aureus in the dairy products. Therefore, this study aimed to study variation in bacterial isolation percentage and the bacterial counts in raw milk and locally produced soft cheese in local markets in Baghdad. A total of 150 samples of raw milk and local soft cheese (75 for each) were collected from different regions of Baghdad city from October 2020 to July 2021 to study the evaluation of bacterial contamination. The isolation percentage of total *coliform*, *Fecal coliform*, *Escherichia coli* and *Staphylococcus aureus* in the raw milk were 82, 69, 54 and 42%, respectively. While in the soft cheese, the isolation percentage for *coliform*, *Fecal coliform*, *Escherichia coli* and *Staphylococcus aureus* were 90, 74, 60 and 45%, respectively. Furthermore, high percentages of bacterial isolation were recorded during summer. The recorded data showed significant (P<0.05) variation for both raw and soft cheese according to months, and soft cheese had a higher isolation percentage than the raw milk samples. The average values of bacterial count values that were isolated from October to February in the raw milk of the total *coliform*, fecal *coliform*, *E. coli* and *Staph aureus* were 5.57, 4.25, 3.77 and 2.94 cfulog$_{10}$/mL respectively, which were recorded during the cold months. While the recording of the average values from March to July as hot months were 6.02, 5.02, 5.22 and 3.23 CFU log10/mL, respectively. The average bacterial values in the soft cheese were 6.02, 5.03, 4.97 and 3.67cfu log$_{10}$ /g, respectively, from October to February and were significantly (P<0.05) less than the summer from March to July, which recorded 7.17, 6.32, 5.01 and 4.15cfu log$_{10}$/g respectively. The high contamination found in the soft cheese and during hot months compared with raw milk and cold months, respectively, is a sign of unsanitary manufacturing conditions such as post-process contamination, high temperature in summer, and lack of refrigeration during long-distance transportation.

Keywords: Raw Milk, Soft Cheese, Coliform, *Staphaureus*, Variation, Contamination

1. Introduction

Milk and cheese were recognized as highly nutritious food due to their high protein, fat and minerals (calcium, phosphorus, iron, and vitamins) (1). These nutrients make it a significant source for infants, newborns, and adults (2). Bacteriological analysis indicates the quality and safety of products that comply with standards, specifications and regulatory requirements (3). Accidental food contamination through the handlers could result in many kinds of bacteria, especially *Staphylococcus aureus*, in the raw milk or its products (4). Subclinical mastitis is also a source of *Staphyaureus*, which causes food poisoning (5). *E. coli* is an enteric pathogen, and it also could be a source of public health concern that might be contaminated cheese around the world (6). The total coliform term refers to the large group of Gram-negative rod-shaped bacteria, thermotolerant coliform, and faecal origin bacteria isolated from the environment (7). Coliforms are opportunistic pathogens that cause a wide range of infections, while many others are part of the normal intestinal flora (8). These organisms in the milk and milk products indicated the improper handling and/or unsanitary production of milk and milk utensils (9). Fecal coliforms cover a small percentage of the total coliform population. Many studies indicate that *E. coli* is the primary coliform representing the fecal environment (10). Raw milk can be contaminated in two ways: internal contamination or endogenous contamination when an animal is infected with one of the microorganisms and transferred to the blood (systemic infection) or by infecting the udder; these microorganisms may be transferred into the raw milk (11). The second way is external or exogenous contamination when milk is contaminated during or after the collection process by faeces, the exterior of the udder and teats, skin and other environmental contamination (11). The contamination of raw milk by spoilage and pathogenic microorganisms can be affected by many factors, including the health status of dairy animals, hygienic milking process, storage conditions, environment, farm management practices, location and season variation (12). Thus, this study aimed to study variation in bacterial isolation percentage and the bacterial counts in raw milk and locally produced soft cheese in local markets in Baghdad.

2. Materials and Methods

2.1. Sampling

A total of 150 samples (75 for each raw milk and locally produced soft cheese) were collected from six regions of Baghdad. The three regions of Rusafa district were (Al-Saadar city, Fadhiliya and Al-Sadria) and the three regions of Karch district were (Abu Ghraib, Radwaniyah and Al-Shiela). All samples (raw milk and locally produced cheese) were collected randomly from local markets and homemade in Baghdad during cold and hot months. The samples were kept in the ice box using sterilized bottles and polyethene bags. Then samples were transported to the Veterinary public health laboratory/ College of the Veterinary Medicine/ University of Baghdad for further investigation. Samples were analyzed from October to February 2020 as (cold months) and from March to July 2021 as (hot months).

2.2. Preparation of Samples for Bacterial Isolation

Seventy-five raw milk samples were collected in aseptic conditions. One ml sample was transferred aseptically into a screw-capped test tube containing 9 mL of sterile 0.1% peptone water (Himedia). Samples were serially diluted up to 10^{-7} using 10-fold serial dilutions (7). Seventy-five soft cheese samples were purchased from a local supermarket and homemade; ten grams of each prepared sample were transferred into sterile warmed water (40-45˚C) 90 mL of (2% sodium citrate) was purchased from BDH (England), homogenized for (2-5) minutes with a homogenizer (Sigma Aldrich/ Germany). Ten-fold dilution (10^{-1}-10^{-7}) was prepared for the different bacterial enumerations (13).

2.3. Isolation and Bacterial Counts

0.1 µL of tested sample from each dilution (10^{-1} to 10^{-7}) that had been previously prepared from both raw milk and soft cheese were put in the empty sterile plate, and 10 to 15 mL of sterile VRB agar (Himedia) incubated at 37 ˚C and 44.5-45.5 ˚C for total and fecal coliform respectively for 24 hours. Dark red colonies were considered typical coliforms on the VRB agar (14). Eosin methylene blue agar (EMB) (Himedia) was used to isolate, and identification of *E. coli* using a sterile L-shaped bent glass; the plates were allowed to dry and inverted and incubated for 24-48 hours at 37 ˚C, and colonies with a green metallic sheen were considered positive result table 1. Mannitol salt agar (Himedia) was used to isolate presumptive coagulase-positive staphylococci in the milk and incubated at 37 ˚C for 24 hours (15). Some *Staph aureus* isolates were able to ferment mannitol sugar and created large golden colonies surrounded by broad yellow zones (16). Baird parker agar supplemented with 5% egg yolk tellurite (DIREVO/Germany) emulsion was used to isolate *Staph aureus*. The samples were spread evenly on the soiled agar using a sterile L-shaped bent glass. The plates were allowed to dry. After that, plates were inverted and incubated at 37 ˚C for 24-48 hours (17). *Staph aureus* colonies appeared as dark grey-black shiny convex, narrow and white, surrounded by a zone of clearing colonies (18). All positive *Staph aureus* and *E.coli* isolates were subjected to biochemical and serological tests such as catalase, DNase, Gram stain, Dry spot *Staph aureus* and Latex mast staph. *Staph aureus* and total coliform produced a negative result of the oxidase test, while *E. coli* and fecal coliform produced positive results as a purple colour (19). Total coliform, fecal coliform, *Staph aureus* and *E. coli* produced positive results for the catalase test as a bubble production because they all produced catalase enzyme (16). *Staph aureus* gave positive results as agglutination to the dry spot and latex mast staph (20)*;* *S. aureus* produced DNase on the DNase agar as a yellow zone surrounding the colonies (16). *Staph aureus* is a Gram-positive bacteria recognized by gram's staining method as a coccus and violet-coloured due to the ability of these bacteria to absorb crystal violet (21), as shown in table 1.

2.4. Statistical Analysis

Statistical data analysis was performed using SAS (Statistical Analysis System - version 9.1). Two-way analysis of variance (ANOVA) and Least significant differences (LSD) post hoc tests were performed to assess significant differences among means for more than two groups, whereas an independent *t*-test was used for two groups. The *Chi*-square test was used to assess the differences between the two proportions. $P<0.05$ is considered statistically significant (22).

Table 1. The cultural, biochemical and serological characterization of *Staphylococcus aureus,* total coliform, fecal coliform, and *E. coli* isolates from raw milk and locally produced soft cheese

	Microorganisms				
	S. aureus		**Total coliform**	**Fecal coliform**	***E. coli***
Media	Baird-Parker agar supplement with egg yolk emulsion	Mannitol salt agar	VRB agar	VRB agar	EMB agar
The description of the culture media	black colonies with Shining colour	Colonies with golden yellow	Colonies with dark red	Colonies with dark red	Colonies with dark colour and a green metallic sheen
Gram stain	**Positive**		**Negative**	**Negative**	**Negative**
Catalase	Catalase positive (bubbles)		Positive	Positive	Oxygen bubble
DNase	The clear zone around the colony				
Oxidase	Negative		Negative	Positive	Positive
Dry spot *staph. aureus*	Agglutination				
Latex mast staph	Agglutination				

3. Results and Discussion

The differences in the isolation percentage of total coliform, fecal coliform, *E. coli* and *Staph aureus* in the raw milk and locally soft cheeses obtained from various local retail markets and homemade in Baghdad city are shown in tables 2 and 3. The results reported that there were significant differences (P<0.05) between raw milk and soft cheese samples; among the 75 raw milk samples tested, 82% (62/75) of samples were contaminated with coliforms. The incidence of fecal coliform in the raw milk was (69%; 52/75). *E. coli* bacteria 54% (41/75) and *Staph aureus* 42% (32/75); among the 75 cheese samples tested, about 90% (67/75) were contaminated with coliforms, 74% fecal coliform (56/75), 60% of samples contaminated with *E. coli* (45/75). The months' isolation percentage of total coliform, fecal coliform, *E. coli* and *S. aureus* showed a significant (P<0.05) variation in both raw milk and soft cheese. The soft cheese had a significantly (P<0.05) higher incidence of total coliform, fecal coliform, *E. coli* and *Staph aureus* (90%, 74%, 60%, and 45%, respectively) than in the raw milk samples (82%, 69%, 54% and 42% respectively) table 2 and 3. Therefore, the soft cheese had a high isolation percentage and was more contaminated than raw milk, as shown in table 4.

Table 2. The incidence and isolation percentage (%) of total coliform, fecal coliform, *E. coli* and *S. aureus* isolated from raw milk samples in different regions of Baghdad according to the months

Months	Year	No of sample	Bacteria			
			Total coliform	**Fecal coliform**	***E. coli***	***S. aureus***
October	2020	8	7/7 (100%)	6/7 (85%)	5/7 (71%)	3/7 (42%)
November		8	6/7 (85%)	5/7 (71%)	4/7 (57%)	3/7(42%)
December		7	6/8 (75%)	5/8 (62%)	4/8 (50%)	3/8 (37%)
January	2021	8	6/8 (75%)	5/8 (62%)	4/8 (50%)	3/8 (37%)
February		7	5/8 (62%)	4/8 (50%)	3/8 (37%)	3/8 (40%)
March		7	5/7 (71%)	4/7 (57%)	3/7 (42%)	3/7 (42%)
April		7	6/7 (85%)	5/7 (71%)	4/7 (57%)	3/7 (42%)
May		7	6/7 (85%)	5/7 (71%)	4/7 (57%)	4/7 (57%)
June		8	7/8(87%)	6/8 (75%)	4/8 (50%)	3/8 (37%)
July		8	8/8 (100%)	7/8 (87%)	6/8 (75%)	4/8 (50%)
Total		75	62/75 (82%)	52/75 (69%)	41/75 (54%)	32/75 (42%)
P-value			0.24	0.43	0.51	0.68

Table 3. The incidence and isolation percentage (%) of total coliform, fecal coliform, *E. coli* and *S. aureus* isolated from soft cheese samples in different regions of Baghdad according to the months

Months	Year	No of sample	Bacteria			
			Total coliform	**Fecal coliform**	***E. coli***	***S. aureus***
October	2020	8	7/7 (100%)	6/7 (85%)	5/7 (71%)	3/7 (42%)
November		8	6/7 (85%)	6/7 (85%)	4/7 (57%)	3/7(42%)
December		7	7/8 (87%)	6/8 (75%)	5/8 (62%)	4/8 (50%)
January	2021	8	6/8 (75%)	5/8 (62%)	4/8 (50%)	3/8 (37%)
February		7	6/8 (75%)	5/8 (62%)	4/8 (50%)	4/8 (40%)
March		7	6/7 (85%)	5/7 (71%)	4/7 (57%)	2/7 (28%)
April		7	6/7 (85%)	5/7 (71%)	4/7 (57%)	3/7 (42%)
May		7	7/7 (100%)	5/7 (71%)	4/7 (57%)	4/7 (57%)
June		8	8/8(100%)	6/8 (75%)	5/8 (62%)	4/8 (50%)
July		8	8/8 (100%)	7/8 (87%)	6/8 (75%)	4/8 (50%)
Total		75	67/75 (90%)	56/75 (74%)	45/75 (60%)	34/75 (45%)
P-value			0.18	0.33	0.46	0.71

Table 4. Relationship between bacterial counts in the milk and cheese samples

Samples	No of sample	Total coliform	Fecal coliform	*E. coli*	*Staph aureus*
Raw milk	75	62 (82%)	52 (69%)	41 (54%)	32 (42%)
Soft chees	75	67 (90%)	56 (74%)	45 (60%)	34 (45%)
Chi-square value		1.38	0.52	0.43	0.10
P-value		0.23	0.46	0.50	0.74

There were significant differences ($P<0.05$) in the total coliform between hot and cold months for raw milk and soft cheese. In cold months the total coliforms were less than in hot months for both samples table 5. The average values of the total coliforms in the raw milk from October to February were 5.57cfulog$_{10}$ /ml during cold months and 6.02 CFU log$_{10}$/ml during hot months. In the soft cheese samples, the average values of total coliform were 7.17cfulog$_{10}$ /g during hot months from March to July, while 6.02cfulog$_{10}$/g during cold months (Table 5). The total coliform counts were recorded more during hot months than the cold months ($P<0.05$) for both raw milk and soft cheese samples. Soft cheese samples were more contaminated with coliform than the raw milk samples, as shown in table 5.

The average value of the fecal coliforms counts from October to February in the soft cheese samples was more than raw milk (5.03cfulog$_{10}$ /g) during cold months and (6.32cfulog$_{10}$ /g) from March to July during hot months (Table 6). While the counts were 4.25 and 5.02 cfulog$_{10}$/mL during cold and hot months, respectively, in the raw milk. According to the months, there were significant differences significantly ($P\leq0.05$) between fecal coliform in both raw milk and soft cheese samples. Soft cheese samples were significantly more contaminated with fecal coliform than raw milk ($P\leq0.05$), and the higher value was in July, as shown in table 6.

E. coli counts with average values in the raw milk from October to February (3.77 cfulog$_{10}$/mL) during cold months were lower than in the hot months from March to July (5.22 cfulog$_{10}$ /mL) (Table 7). The average counts of soft cheese samples from October to February were (4.97cfulog$_{10}$ /g) during cold months, and from March to July were (5.01cfulog$_{10}$ /g) during hot months. There were significant differences ($P<0.05$) in the *E. coli* counts between raw milk and soft cheese samples and among the months. Soft cheese samples were with higher counts than raw milk samples significantly ($P<0.05$). *E. coli* counts during cold months were lower than during the hot months significantly ($P<0.05$) (Table 7).

The average value of *Staphaureus* presumptive counts in the raw milk from October and February was (2.94 CFU log$_{10}$/ml) during cold months, and from March to July, was (3.23 CFU log$_{10}$/ml) during hot months, and the higher value was in July (Table 8). The average counts for the soft cheese samples from October to February were (3.67 cfulog$_{10}$ /g) during cold months, and from March and July were (4.15cfulog$_{10}$ /g) during hot months, and the higher was in July (Table 8). There was a significant difference ($P<0.05$) among the counts of *Staphaureus* between hot and cold months and between raw milk and soft cheese samples. The results showed more *Staphaureus* counts in soft cheese than in raw milk. *Staph aureus* counts during hot months were significantly higher ($P<0.05$) than in cold months in both raw milk and soft cheese samples, and the highest value was in July (Table 8).

Table 5. Total coliform counts in the examined raw milk and soft cheese samples collected from different regions of Baghdad according to the months

Year	Samples / Months	Raw milk (Mean±SE) (CFU log_{10} /mL)	Soft cheese (Mean±SE) (CFU log_{10} /g)
2020	October	$5.00^{B}±0.35^{cd}$	$6.11^{A}±0.44^{cd}$
	November	$4.27^{B}±0.40^{d}$	$6.30^{A}±0.56^{cd}$
	December	$6.23^{A}±0.47^{a}$	$6.78^{A}±0.52^{bc}$
2021	January	$6.23^{A}±0.52^{a}$	$5.09^{B}±0.53^{e}$
	February	$6.16^{A}±0.47^{ab}$	$5.84^{A}±0.49^{de}$
	Average	$5.57^{B}±0.44^{bc}$	$6.02^{A}±0.50^{bc}$
	March	$5.42^{B}±0.42^{bc}$	$6.27^{A}±0.24^{cd}$
	April	$5.30^{B}± 0.42^{c}$	$6.23^{A}±0.39^{cd}$
	May	$6.30^{B}±0.47^{a}$	$7.88^{A}±0.53^{a}$
	June	$6.70^{B}±0.48^{a}$	$7.30^{A}±0.61^{ab}$
	July	$6.42^{B}±0.48^{a}$	$7.73^{A}±0.61^{a}$
	Average	$6.02^{B}±0.45^{ab}$	$7.17^{A}±0.47^{ab}$
	LSD	0.80	

Means with a different small letter in the same column are significantly different (P<0.05)
Means with a different capital letter in the same row are significantly different (P<0.05)

Table 6. Fecal coliform counts in the examined raw milk and soft cheese samples collected from different regions of Baghdad according to the months

Year	Samples / Months	Raw milk (Mean±SE) (CFU log_{10} /mL)	Soft cheese (Mean±SE) (CFU log_{10} /g)
2020	October	$4.14^{A}±0.36^{c}$	$4.29^{A}±0.27^{d}$
	November	$4.37^{A}±0.33^{bc}$	$4.31^{A}±0.35^{d}$
	December	$4.29^{B}±0.30^{c}$	$5.42^{A}±0.34^{bc}$
2021	January	$4.11^{B}±0.46^{c}$	$5.34^{A}±0.41^{bc}$
	February	$4.34^{B}±0.31^{bc}$	$5.78^{A}±0.20^{bc}$
	Average	$4.25^{B}±0.35^{bdc}$	$5.03^{A}±0.31^{cd}$
	March	$4.38^{B}±0.38^{bc}$	$5.30^{A}±0.33^{c}$
	April	$5.32^{B}±0.23^{a}$	$6.29^{A}±0.22^{b}$
	May	$5.98^{A}±0.33^{a}$	$6.29^{A}±0.24^{b}$
	June	$5.21^{B}±0.31^{ab}$	$6.29^{A}±0.60^{b}$
	July	$4.25^{B}±0.21^{c}$	$7.45^{A}±0.60^{a}$
	Average	$5.02^{B}±0.29^{ab}$	$6.32^{A}±0.39^{ab}$
	LSD	0.91	

Means with a different small letter in the same column are significantly different (P<0.05)
Means with a different capital letter in the same row are significantly different (P<0.05)

Table 7. *E. coli* counts in the examined raw milk and soft cheese samples collected from different regions of Baghdad according to the months

Year	Samples / Months	Raw milk (Mean±SE) (CFU log_{10} /mL)	Soft cheese (Mean±SE) (CFU log_{10} /g)
2020	October	$3.33^{B}±0.27^{c}$	$5.37^{A}±0.47^{a}$
	November	$3.38^{B}±0.28^{c}$	$4.38^{A}±0.44^{b}$
	December	$3.38^{B}±0.30^{c}$	$4.39^{A}±0.38^{b}$
2021	January	$4.38^{A}±0.28^{b}$	$4.43^{A}±0.42^{b}$
	February	$4.41^{B}±0.23^{b}$	$5.84^{A}±0.34^{a}$
	Average	$3.77^{B}±0.27^{bc}$	$4.97^{A}±0.41^{b}$
	March	$4.39^{A}±0.23^{b}$	$4.35^{A}±0.36^{b}$
	April	$5.30^{A}±0.24^{a}$	$4.35^{B}±0.35^{b}$
	May	$5.39^{A}±0.40^{a}$	$5.73^{A}±0.45^{a}$
	June	$5.70^{A}±0.41^{a}$	$5.29^{A}±0.50^{a}$
	July	$5.32^{A}±0.31^{a}$	$5.34^{A}±0.51^{a}$
	Average	$5.22^{A}±0.32^{ab}$	$5.01^{A}±0.43^{ab}$
	LSD	0.85	

Means with a different small letter in the same column are significantly different (P<0.05)
Means with a different capital letter in the same row are significantly different (P<0.05)

Table 8. *Staph aureus* counts in the examined raw milk and soft cheese samples collected from different regions of Baghdad according to the months

Year	Samples / Months	Raw milk (Mean±SE) (CFU log_{10} /mL)	Soft cheese (Meam±SE) (CFU log_{10} /g)
2020	October	$2.30^{B}±0.29^{c}$	$3.66^{A}±0.27^{ab}$
	November	$3.43^{A}±0.14^{b}$	$4.35^{A}±0.26^{ab}$
	December	$3.42^{A}±0.19^{b}$	$3.27^{A}±0.31^{b}$
2021	January	$3.38^{A}±0.30^{b}$	$3.37^{A}±0.30^{cd}$
	February	$2.26^{B}±0.25^{c}$	$3.27^{A}±0.24^{d}$
	Average	$2.94^{B}±0.23^{bc}$	$3.67^{A}±0.27^{ab}$
	March	$2.37^{B}±0.26^{c}$	$3.70^{A}±0.20^{cd}$
	April	$3.30^{A}±0.25^{b}$	$3.33^{A}±0.28^{cd}$
	May	$3.39^{B}±0.30^{b}$	$4.05^{A}±0.28^{bc}$
	June	$2.20^{A}±0.29^{c}$	$4.42^{B}±0.31^{ab}$
	July	$4.92^{A}±0.22^{a}$	$4.84^{A}±0.22^{a}$
	Average	$3.23^{B}±0.26^{ab}$	$4.15^{A}±0.25^{ab}$
	LSD	0.62	

Means with a different small letter in the same column are significantly different ($P<0.05$)
Means with a different capital letter in the same row are significantly different ($P<0.05$)

The prevalence of total coliform, fecal coliform, *Staphaures*, and *E. coli* showed significant variation in the months, which was higher in hot months than in the cold months (Tables 2 and 3). This could be due to high ambient temperature and fewer refrigeration conditions that encourage the growth and multiplication of bacteria (23).The isolation percentage was less than Ahmed (24), who recorded that the percentage of *Staphaureus* in the soft cheese was (73.33%) from the 30 examined samples. The percentage of *Staph aureus* was lowest compared to coliform and *E. coli*, as shown in tables 2 and 3, and this could be due to the fact that the source of milk was from healthy animals and not infected with mastitis, which led to an increase in *Staph aureus* percentage, and that the source of bacteria was mainly from fecal and water contamination of milk (5). In Turkey, Honish, Predy (25) found that about 4% of soft cheeses are contaminated with coliform. The increased contamination of soft cheese samples compared to raw milk samples (Table 4) agree with Drudy, Mullane (26), who indicated that the cheese contamination was caused by low-quality raw milk that was used for cheese manufacturing, processing under unhygienic conditions, and an unsuitable starting culture for fermentation during ripening. Subclinical mastitis, caused by *E. coli*, can also cause sporadic high coliform levels (27). Fusco, Quero (28) reported that *Staphaureus* was recorded at 54% in the raw milk samples; while Jørgensen, Mathisen (29) found that the contamination with *Staphaureus*was reported in 75% of bovine raw milk samples. Furthermore, El-Diasty and El-Kaseh (30) indicated that the mean total coliform $7.0×10^{6}$ was CFU/ml for raw milk samples. The current study's variances include varied healthy practices during milking, weather fluctuations, herd cleanliness, dirty water, geographical distribution, poorly kept washed equipment, and unhealthy milking processes (25). The presence of coliform contamination in raw milk and milk products indicated sloppy manufacturing and/or improper handling of milk or milk utensils (31). Total coliform and fecal coliform have probably gotten more attention than most other types of bacteria from the public health aspect because of their usefulness as indicator organisms for anticipating unsanitary conditions during different milk production, handling and processing. They are responsible for the spoilage of milk and milk products, acting as good indicators for the presence of other pathogens (10). The high contamination of raw milk samples occurs due to poor sanitation, storage, production times and temperature

(32). This result agreed with Muhammad, Altaf (33), who found that the number of coliforms in raw milk was higher during warm months. The high counts of *E. coli* in the soft cheese compared to the raw milk result agree with Hassan and Afify (34), who reported that the incidence of *E. coli* were (6%) and (86%) in the raw milk and cheese that collected from different shops and farmers houses in Egypt. Because *E. coli* bacteria can grow in a variety of substrates and use a variety of carbohydrates and other organic compounds, the presence of high *E. coli* counts in the raw milk and its products degrade the quality of the final product, making it unmarketable during storage or even unfit for human consumption, leading to economic losses, particularly during summer season (35). The significant differences ($P<0.05$) among the counts between raw milk and soft cheese and between hot and cold months in the soft cheese samples (Table 8) agree with Fadaei (36) who reported that the number of *S. aureus* in the summer more than those in winter in the raw cow milk. The contamination with a high incidence rate *of S. aureus* with mean log counts of 6.930 pointed out the significant amount of contamination in locally produced buffalo soft cheese samples, which could provide a public health risk to Iraqi consumers (37). The presence of *S. aureus* in cheese usually means that milk had been contaminated by poorly udders or external surfaces of dairy animals, or by infected, unclean hands of dairy workers, raw milk contamination used in cheese manufacturing, or by their sneezing and coughing (38). Insanitary equipment, failure to wash the udders before milking, no mastitis investigations, unhealthy milking vessels and milk containers or tanks, long delivery times, high temperature, lack of farmers' education, and poor personnel hygiene all of these was contributing to the contamination with an increased number of bacteria and contaminated the milk samples (36). Yadav, Singh (39) indicated that the total coliform and fecal coliforms were 0-1000 and 20-100 CFU/100ml respectively in summer, while 2-490 and 2-50 CFU/100ml respectively in winter and explained that the high temperature was a crucial factor that enhances growth and proliferation of bacteria. The climate significantly impacts bacterial growth; therefore, this study focused on the isolation and detection of bacterial counts that occur during hot and cold months of local products sold in Baghdad.

During hot months, the high contamination of raw milk and soft cheese with coliform, *E. coli* and *Staph aureus* bacteria occurs due to high temperature and lack of refrigeration. Low-quality raw milk used in cheese manufacturing and processing under unhygienic conditions leads to more contaminated cheese than raw milk. The high contamination occurs during the hot months compared to cold months could be attributed to unsanitary manufacturing conditions such as post-processing contamination, pasteurization failure, the high ambient temperature in the summer and the lack of refrigeration in the long distance during milk transportation. Therefore, hygienic practices are required during the raw milk collection, transportation, and storage, particularly during the hot months.

Authors' Contribution

Study concept and design: M. A. R. A.
Acquisition of data: Z. S. K.
Analysis and interpretation of data: M. A. R. A.
Drafting of the manuscript: M. A. R. A.
Critical revision of the manuscript for important intellectual content: Z. S. K.
Statistical analysis: Z. S. K.
Administrative, technical, and material support: Z. S. K.

Ethics

The study was approved by the Research Ethics Committee of the University of Kufa, Kufa, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The Department of Public Health at the Veterinary Medicine College, University of Baghdad, Iraq, and all its employees deserves special recognition.

References

1. McGee H. Cheese on Food and Cooking. Revised ed. Scribner; 2004.
2. Torres-Vitela M, Mendoza-Bernardo M, Castro-Rosas J, Gomez-Aldapa C, Garay-Martinez L, Navarro-Hidalgo V, et al. Incidence of Salmonella, Listeria monocytogenes, Escherichia coli O157 :H7, and staphylococcal enterotoxin in two types of Mexican fresh cheeses. J Food Prot. 2012;75(1):79-84.
3. Varga L. Microbiological quality of commercial dairy products.2007.
4. Aung MS, San T, Aye MM, Mya S, Maw WW, Zan KN, et al. Prevalence and genetic characteristics of Staphylococcus aureus and Staphylococcus argenteus isolates harboring Panton-Valentine leukocidin, enterotoxins, and TSST-1 genes from food handlers in Myanmar. Toxins. 2017;9(8):241.
5. Sahu B, Mukherjee R, Ajay K, Amit K, Jyoti S. Prevalence of coagulase gene positive Staphylococcus aureus bovine mastitis in three distinct geoclimatic regions of India. Buffalo Bull. 2014;33(2):208-14.
6. Kousta M, Mataragas M, Skandamis P, Drosinos EH. Prevalence and sources of cheese contamination with pathogens at farm and processing levels. Food control. 2010;21(6):805-15.
7. Marjan S, Das KK, Munshi SK, Noor R. Drug-resistant bacterial pathogens in milk and some milk products. Nutr Food Sci. 2014.
8. Katouli M, Melin L, Jensen-Waern M, Wallgren P, Möllby R. The effect of zinc oxide supplementation on the stability of the intestinal flora with special reference to composition of coliforms in weaned pigs. J Appl Microbiol. 1999;87(4):564-73.
9. El Zubeir IE, Ahmed MI. The hygienic quality of raw milk produced by some dairy farms in Khartoum State, Sudan. Res J Microbiol. 2007;2(12):988-91.
10. Dhanashekar R, Akkinepalli S, Nellutla A. Milk-borne infections. An analysis of their potential effect on the milk industry. Germs. 2012;2(3):101.
11. Verraes C, Vlaemynck G, Van Weyenberg S, De Zutter L, Daube G, Sindic M, et al. A review of the microbiological hazards of dairy products made from raw milk. Int Dairy J. 2015;50:32-44.
12. Muehlhoff E, Bennett A, McMahon D. Milk and dairy products in human nutrition: Food and Agriculture Organization of the United Nations (FAO); 2013.
13. Richardson GH. Standard Methods for the Analysis of Dairy Products. 1985.
14. Mansour AMA, Ishlak AMM, Haj-Saeed BA. Evaluation of bacterial contamination on local and imported mutton in meat markets in Benghazi-Libya. Int J Agric Sci. 2019;4.
15. Brown AE, Heidi S. Microbiological Applications. McGRAW-Hill Companies. 2005;35:217-24.
16. Brooks GF, Butel JS, Morse SA. Medical microbiology. United States, 25th. 2006.
17. Pisano MB, Fadda ME, Deplano M, Corda A, Cosentino S. Microbiological and chemical characterization of Fiore Sardo, a traditional Sardinian cheese made from ewe's milk. Int J Dairy Technol. 2006;59(3):171-9.
18. O'Brien M, Hunt K, McSweeney S, Jordan K. Occurrence of foodborne pathogens in Irish farmhouse cheese. Food Microbiol. 2009;26(8):910-4.
19. Liu J-K, JURTSHUK JR P. N, N, N′-N′-Tetramethyl-p-Phenylenediamine-Dependent Cytochrome Oxidase Analyses of Bacillus Species. Int J Syst Evol. 1986;36(1):38-46.
20. Kumurya A. Malfunction of Agglutination Test to Identify Methicillin-Resistant Staphylococcus aureus Strains (MRSA). International Journal of Bioinformatics and Biomedical Engineering.2015;1(1):1-6.
21. Biswas B, Basu P, Pal M. Gram staining and its molecular mechanism. Int Rev Cytol. 1970;29:1-27.
22. Cary NC. SAS. SAS/STAT Users Guide for Personal Computer. Release 9.13. USA: SAS Institute, Inc; 2010.
23. Bradley AJ. Bovine mastitis: an evolving disease. Vet J. 2002;164(2):116-28.
24. Ahmed AJ. Evaluation of the bactericidal effect of Nisin and/or Potassium sorbate and Sodium chloride on the viability of Staphylococcus aureus in soft cheese: Zina Saab Khudhir and Adnan Jawad Ahmed. Iraqi J Vet Med. 2017;41(1):55-9.
25. Honish L, Predy G, Hislop N, Chui L, Kowalewska-Grochowska K, Trottier L, et al. An outbreak of E. coli O157: H7 hemorrhagic colitis associated with unpasteurized gouda cheese. Can J Public Health. 2005;96(3):182-4.
26. Drudy D, Mullane N, Quinn T, Wall P, Fanning S. Enterobacter sakazakii: an emerging pathogen in powdered infant formula. Clin Infect Dis. 2006;42(7):996-1002.

27. Torkar KG, Teger SG. The microbiological quality of raw milk after introducing the two day's milk collecting system. Acta Agric Slov. 2008;92(1):61-74.
28. Fusco V, Quero GM, Morea M, Blaiotta G, Visconti A. Rapid and reliable identification of Staphylococcus aureus harbouring the enterotoxin gene cluster (egc) and quantitative detection in raw milk by real time PCR. Int J Food Microbiol. 2011;144(3):528-37.
29. Jørgensen HJ, Mathisen T, Løvseth A, Omoe K, Qvale KS, Loncarevic S. An outbreak of staphylococcal food poisoning caused by enterotoxin H in mashed potato made with raw milk. FEMS Microbiol Lett. 2005;252(2):267-72.
30. El-Diasty EM, El-Kaseh R. Microbiological monitoring of raw milk and yoghurt samples collected from El-Beida city. Arab J Biotech. 2009;12(1):57-64.
31. El-Bakri J, El-Zubeir IE. Chemical and microbiological evaluation of plain and fruit yoghurt in Khartoum State, Sudan. Int J Dairy Sci. 2009;4(1):1-7.
32. Kaldes YT. Microbiological examination of soft cheeses manufactured in Minia city. Assiut Vet Med J. 1997;38(75):39-47.
33. Muhammad K, Altaf I, Hanif A, Anjum A, Tipu M. Monitoring of hygienic status of raw milk marketed in Lahore City, Pakistan. J Anim Plant Sci. 2009;19(2):74-7.
34. Hassan G, Afify SI. Occurrence of some pathogenic microorganisms in kareish cheese and their public health significance. J Vet Med Res. 2008;18(1):142-50.
35. Megawer A, Hassan G, Meshref A, Elnewery H. Prevalence of Escherichia coli in Milk and Some Dairy Products in Beni-Suef Governorate, Egypt. J Vet Med Res. 2020;27(2):161-7.
36. Fadaei A. Bacteriological quality of raw cow milk in Shahrekord, Iran. Vet World. 2014;7(4):240-3.
37. Khudhir Z. The synergistic effect of pH and sodium citrate on the bacteriocidal activity of Nisin against Staph aureus. Int J Vet Sci. 2019;8(1):49-53.
38. Salem H, El-Attar L, Omran E. Microbiological assessment of some parameters of Kariesh cheese sold by supermarkets and street vendors in Alexandria, Egypt. J High Inst Public Health. 2016;46(2):77-85.
39. Yadav N, Singh S, Goyal SK. Effect of seasonal variation on bacterial inhabitants and diversity in drinking water of an office building, Delhi. Air, Soil Water Res. 2019;12.