

Incidence of *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* in Fresh White Cheese in Gaza City Markets

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ABSTRACT— *Listeria monocytogenes* has gained worldwide interest because it cause food borne illnesses. A descriptive study was performed in the period between October 2004 and October 2005 on one hundred and seventy four samples of fresh white cheese (114 made by farmers and 60 samples made in factories) collected from street vendors and suppliers of six licensed dairy factories to investigate the incidence of *L. monocytogenes*, and other pathogenic bacteria in fresh white cheese in Gaza. Results showed that, *L. monocytogenes* was not detected at all, while, *Salmonella* was found in four samples of "Traditional cheese". which exceed limits of Palestinian Standard 46.5% for *Staph. aureus*, 79% for *Coliform*, 39.5% for *E. coli*, 51.3% for *Yeast*, and 12.8% for *Molds*. Differences between percentages of samples were statistically significant for all tested microorganisms ($P < 0.05$) except for *Salmonella* and *Mold*. The results proved that traditional cheese, in particular, in Gaza were highly contaminated and usually exceeded the Palestinian Standard.

Keywords--- Fresh White Cheese, Listeriosis, *L. monocytogenes*, Gaza

1. INTRODUCTION

Palestinian fresh white cheese is a kind of soft cheese made of cows' and ewes' milk, by factories or by farmers, eaten fresh and has rich nutrient contents. Palestinian Annual Report (2004) detected that of 1203 samples of milk and milk products, 440 samples (36.6 %) did not comply with microbiological criteria of Palestinian Standards (PS). Also, a study made in Agriculture Research Center, Giza, Egypt (2003) showed that of 1750 samples of fresh white cheese, about 90% of samples were contaminated by harmful bacteria .

Raw or pasteurized milk and cheese are frequently implicated as vehicles of transmission of pathogenic bacteria and with outbreaks all over the world (Flowers et al., 1992). Therefore, FDA (2005) advises that hands, cutting boards, counters, knives and other utensils should be washed thoroughly. Besides, good manufacturing practice should be employed at all stages of production and handling, especially with production of raw milk at the farm to the purchase of the cheese by the consumer. The majority of such outbreaks results from unpasteurized, improperly-pasteurized milk, insufficient hygiene conditions in these places or unsuitable conditions outside the environment. California Department of Food and Agriculture CDFA (2002) reported that, *Listeria* and *Salmonella* cannot be detected by sight, taste or smell, where it can cause severe illness to infants, the elderly and those with impaired immune systems. Raw milk soft cheeses can cause several serious infectious diseases including Listeriosis, Brucellosis, Salmonellosis and Tuberculosis (FDA, 2005). Johnson et al. (1990 a.b.c) assigned pathogenic organisms to three risk groups: *Salmonella* spp., *L. monocytogenes* and *E. coli* 0157:H7 were considered high-risk organisms and have been linked to food-borne outbreaks and illnesses in contaminated soft cheese.

A study made by Khalil and Bastawrows (1997) in Assiut- Egypt on milk and kareish cheese, pointed out that 1.25% and 2% were positive for *L. monocytogenes* respectively. Another study by El-prince (1999) detected one sample (2%) of Domiati cheese was positive for *L. monocytogenes* . On the other hand, Javadi (1998) analyzed 150 cheese samples that were collected randomly from 30 factories and 20 traditional cheese manufactures; *L. monocytogenes* was not detected. Similarly, Marzocca et al (2004) analyzed 132 samples of soft cheese using presence/absence criterion. Again, *L. monocytogenes* was not found. Copes et al. (2000) studied the diverse cheeses from supermarkets of direct sale to the public. They found that from 35 analyzed cheeses, four strains of *Listeria* were isolated.

Shehata (1999) reported that 18 samples of unpasteurized soft cheese, 41 samples of pasteurized milk and 51 samples of pasteurized soft cheese were analyzed; *L. monocytogene* was detected in 55% of unpasteurized soft cheese samples, while not detected in both pasteurized milk and soft cheese samples. But Lunden et al. (2004) detected that the pasteurization of raw milk did not eliminate the risk of *L. monocytogenes* contamination in dairy products.

The improper storage under high environmental temperature permits growth of *S. aureus* and can stimulate the production of staphylococcal enterotoxin in soft cheese (Araujo et al., 2002). Some strains produce a highly heat-stable protein toxin that causes disease in humans. *S. aureus* is not very resistant to heat and it is destroyed in one or two minutes in boiling water (Trickett, 1997)

But exposure to temperatures as high as 100°C for as long as 30 minutes does not destroy the staphylococcal enterotoxin, making it an even greater threat to public health (Raszl et al., 2001).

Araujo et al. (2002) and Jorgensen et al. (2005) detected that the *S. aureus* was also found in raw milk products. Furthermore, Al-Tahiri (2005) detected *S. aureus* count 5×10^3 (CFU /g) in cheese made by farmers and no contamination in cheese made by modern dairies in Jordan.

A study reported in Egypt by Eid (1997) detected that 45% of examined Domiati soft cheese samples were contaminated with coliforms with a count ranging from 4×10^2 to 4×10^6 (CFU /g) and mean value 2.46×10^5 .

Results obtained by Aly & Galal (2002) showed that the mean total coliform counts were 1.2×10^5 (CFU/g) in fresh Domiati cheese. While, Araujo et al. (2002), detected that the total coliform ranged from 10^3 to 10^6 and the high levels of contamination in soft cheese analyzed ranged from 10^1 to 10^6 , where, 95.5% of samples $>10^2$ (CFU/g) for faecal coliform. On the other hand, Al-Tahiri (2005) detected coliform 3×10^2 in cheese made by farmers and not detected in cheese made by modern dairies. However, Post-pasteurization contamination, manufacturing and handling process, equipment, temperature abuse during transport and storage conditions might result in high levels of pathogenic microorganisms in cheese (Araujo et al., 2002 and Freitas et al., 1993).

Many authors (Flowers et al. 1992; Johnson et al. 1990 a.b.c; Kerr et al. 1996; Rampling, 1996; and Zottola & Smith, 1993) detected that cheese had been the vehicle for several food-borne disease outbreaks. Carrique et al. (2003) showed that the consumption of raw milk products, especially soft cheese, was therefore clearly a risk factor. Furthermore, soft cheeses have been linked to food-borne outbreaks and illnesses caused by *Salmonella*, *L. monocytogenes*, and *E. coli* contamination (FDA, 1998).

However, *L. monocytogenes* was isolated from 11 samples of 375 (2.9%) in turkey and chicken meat in Gaza strip by El-Manama et al. (2005). Copes et al. (2000) studied the diverse cheeses from supermarkets of direct sale to the public, four strains from 35 of *Listeria* were isolated (11.4%). Although, Marzocca et al (2004) *L. monocytogenes* was not found in 132 samples, This finding urges us to investigate the occurrence of *L. monocytogenes* and other pathogenic bacteria in fresh white cheese in Gaza.

2. MATERIAL AND METHODS

2.1 Study area

A descriptive study, the boundary of the research is Gaza Strip which includes five Governorates North Gaza, Gaza, Midzone, Khan-younis and Rafah. The samples collected from Gaza, which have a very crowded place with an area of 72.47 km²; the population is mainly concentrated in the city and beach refugee camp.

2.2 Methods of research

2.2.1 Size of sample

'Sample Size Calculator for Attribute Ratings' was used to calculate the size of samples obtained from traditional farmers and those taken from factories. Moreover, some data were obtained from Preventive Medicine Directorate in Gaza Strip for the percentages of soft cheese samples that are not accepted according to PS collected by health inspectors at the period from 2001 to 2004. need a sample size of 146 as a total, 104 and 41 samples from traditional and factories respectively.

2.2.2 Cheese Samples

174 samples of fresh cheese were collected from suppliers, markets and retailers in Gaza city. 114 traditional cheese samples were collected from street vendors and some retailers in the five main markets in Gaza city (Al-Shejaia, Al-Zawya, Fras, Al-Shati and Al-Shekh Radwan). And sixty samples were collected from main distributors of six licensed dairy factories, four of them in West Bank (Al-Jebreini, Al-Juniadi, Al-Karmel and Al-Maraei) and the other two factories in Gaza strip (Al-Nada and Al-Fardos) from suppliers of six licensed dairy factories in West Bank and Gaza strip. The samples were processed on the day of acquisition. The samples were immediately transported in plastic ice box to the laboratory in retail packages.

2.2.3 Analysis of samples

All samples of soft cheese were transported in an ice box to PHL in Gaza City in retail packages. Microbiological analyses were performed according to Bacteriological Analytical Manual 8th edition of FDA (1995). Each cheese sample was mixed in a blender with saline to prepare a dilution; all counts were exposed as colony forming units per gram cheese (CFU/g). Chemical analyses (moisture, pH, and salt) in soft cheese were tested by method detected by Association of Official Analysis Chemists AOAC (2000).

2.2.4 Compliance of results

According to PS 22/1997, the soft cheese must have the special taste, odor, color, and be free from foreign matter and spoilage and free of pathogenic bacteria. The results of this work will be compared taking into consideration the microbiological criteria of the sample that was taken from the market, which are as follows: the maximum limits of coliform, Yeast (plus *Oidium lactis*), Mold and *S. aureus* "Coagulase positive" 2×10^3 , 2×10^3 , 2×10^2 and 102 cells/g cheese respectively. Hence, the increase of these counts mentioned above is defined as non-complied or not accepted results in this thesis.

2.2.5 Data analysis

Data were entered and tabulated by using Microsoft Excel and SPSS (Statistical Package for the Social Sciences) version eight. The bacteriological contamination percentage was tabulated and the correlations results between factories and traditional soft cheese samples were compared. Frequency tables were constructed for the study variables. P value less than 0.05 is considered statistically significant and it was calculated depending on Chi-Square test. Odds Ratio was used for measurement of risk at 0.95 Confidence Interval (C.I) for statistical significant testing.

All samples were analyzed in PHL, Public Health Laboratory - Gaza. Procedures For detection of T.B.C and *S. aureus* were followed according to the method of FDA (1995). Detection and isolation of coliform groups and isolate of *E. coli* from coliform groups, *Salmonella* species and *Listeria* species were followed according to the method of FDA (1995).

3. RESULTS

L. monocytogenes was not found in any of the samples made in factories or made by farmers in unlicensed places.

T.B.C is a general indicator of microbial contamination of cheese. The total count in traditional cheese ranged between Nil and 3×10^6 (CFU /g cheese) and presented mean value 3.2×10^5 . For cheese made by licensed factories, the range of T.B.C is between Nil and 6×10^5 and the mean value were 6.4×10^4 . Traditional cheese produced by farmers in unlicensed places gave a high T.B.C 52.6% of samples were $\geq 10^5$ (CFU/g) more than cheese made by factories (16.7%). A highly significant difference between the two groups ($P=0.000$).

S. aureus counts in traditional cheese samples were ranged between Nil and 4×10^4 (CFU /g cheese) and presented mean values 1.7×10^3 . But in cheese made by factories ranged between Nil and 2×10^3 and the present mean value 70. Although, it's not detected in 52.6 % of traditional cheese samples. While not detected in 91.7% of factories samples. 46.5% of traditional cheese samples increased the maximum limit of PS for *S. aureus* ($>10^2$ CFU /g). On the other hand, cheese made in licensed factories had less contamination rate where five out of 60 samples (8.3%) tested increased the maximum limit. However, the variation between two groups is a high statistical significant ($P= 0.000$).

Regarding with total Coliform bacteria, the high contamination percent in traditional cheese samples, which reached 9×10^5 (CFU /g) and presented mean values 5.4×10^4 . On the other hand, coliform in cheese made by licensed factories ranged between Nil and 4×10^5 and the present mean value 1×10^4 . Contamination over the limits established by PS for coliform. 90 out of 114 (79%) traditional cheese samples and 13 out of 60 (21.7%) cheese made by factories were over 2×10^3 (CFU /g). However, the difference between two groups is high statistically significant ($P = 0.000$). Inspection of results recorded revealed that *E. coli* was detected positive in 54 (31%) of 174 soft cheese samples, regarding the traditional cheese, 45 samples were detective positive (39.5%) of 114 samples. On the other hand, nine samples of 60 (15%) were positive. However, the difference was statistically significant ($P = 0.001$).

samples made in licensed factories was free from *Salmonella* spp. But it was isolated from four samples (3.5%) of 114 traditional cheeses. The variation between two groups did not arrive at statistical significant ($P = 0.95$).

However, the statistical analysis of microbiological and chemical tests on traditional soft cheese samples was summarized in the table 1.

Table 1: Statistical analysis results of microbial and chemical tests of traditional cheese

Microbiological (CFU /g)					
Item	No. of Samples	Min.	Max.	Mean	Std. deviation
T.B.C	114	Nil	3×10^6	3.2×10^5	4.5×10^5
S. aureus	114	Nil	4×10^4	1.7×10^3	4.6×10^3
Coliform	114	50	1×10^6	5.4×10^4	1.2×10^5
E. coli	114	-	-	-	-
Salmonella spp.	114	-	-	-	-
Yeast	78	Nil	6×10^4	7.6×10^3	1.3×10^4
Mold	78	Nil	2×10^3	90	2.8×10^2
Chemical					
Moisture %	20	57.7	73.6	63	4.8
NaCl %	34	0.2	6.4	2.65	1.7
pH	79	6	7	6.38	0.36
Fat %	12	12	19	15.3	1.8

Microbial and chemical results of soft cheese made by factories in Table 2 sums up the statistical analysis of microbiological and chemical tests on soft cheese samples.

Table 2: Microbiology and chemical results of soft cheese made by licensed factories

Microbiological (CFU/ /g)					
Item	No. of tested samples	Min.	Max.	Mean	Std. deviation
T.B.C	60	Nil	6×10^5	6.4×10^4	1.2×10^5
S. aureus	60	Nil	2×10^3	70	3.2×10^2
Coliform	60	50	4×10^5	1×10^4	5.3×10^4
E. coli	60	-	-	-	-
Salmonella spp.	60	-	-	-	-
Yeast	48	Nil	1×10^5	8×10^3	2.1×10^4
Mold	48	Nil	Nil	-	-
Chemical					
Moisture %	16	43.3	71	63.9	7.1
NaCl %	38	0.7	6.1	3.87	1.2
pH	47	6	7	6.6	0.34
Fat %	8	12	21	18	2.87

The percentages of acceptable and unacceptable in traditional cheese samples according to microbiological criteria in PS are shown in Figure1.

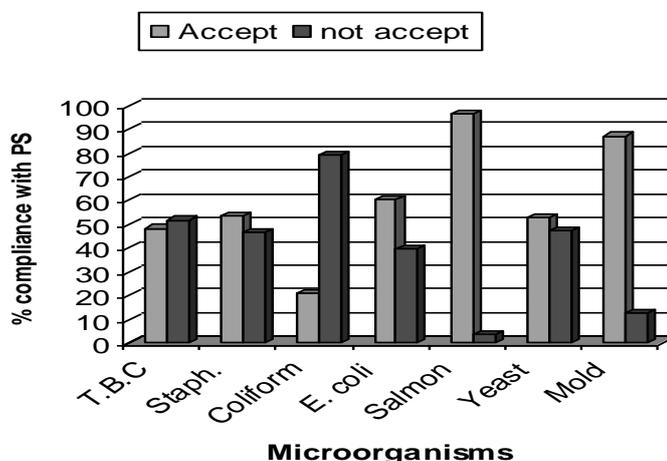


Figure1: Percentages of acceptable and unacceptable traditional soft cheese samples

Microbiological comparison of accepted and unaccepted samples in traditional cheese and factories made, in Table 3.

Table 3: Percentages of accepted and unaccepted samples in traditional cheese and factories made.

Microorganisms	Producers				Statistically analyses P. value
	Farmers		Factories		
	No. of tested samples	Not accepted No.(%)	No. of tested samples	Not accepted No.(%)	
T.B.C	114	59 (51.8 %)	60	10 (16.7 %)	0.000*
<i>S. aureus</i>	114	53 (46.5 %)	60	5 (8.3 %)	0.000*
<i>Coliform</i>	114	90 (79 %)	60	13 (21.7 %)	0.000*
<i>E. coli</i>	114	45 (39.5 %)	60	9 (15 %)	0.001*
<i>Salmonella spp.</i>	114	4 (3.5 %)	60	0 (0 %)	-
Yeast + <i>O. lactis</i>	78	40 (51.3 %)	48	15 (31.2 %)	0.04*
Mold	78	10 (12.8 %)	48	0 (0 %)	-

*: statically significant, - Odds ratio could not be calculated because one cell of table has the value Nil.

4. DISCUSSION

L. monocytogenes was not found in any of the samples made in factories or made by farmers in unlicensed places. Similar results were reported by Marzocca (2004). But they were in disagreement with Copes et al. (2000). The differences in places of cheese-making and the environmental conditions of animals such as silage, manure and bedding materials that may contribute to the microbial load found in/on the udder of the cow.

T.B.C Meanwhile, Aly and Galal (2002) found higher counts in fresh cheese made from raw milk (1.9×10^8), and nearly lower counts with the other sorts made from heat-treated milk (2.8×10^4). Also, AL- Tahiri (2005) found 2×10^4 in cheeses produced by farmers. The increase of T.B.C can be explained by the environmental conditions which allow the growth and multiplication of microorganisms. The variation also, due to low hygiene practices and experience in local factories.

Regarding with *S. aureus*, the results was in agreement with data obtained by Araujo et al. (2002) and Jorgensen et al. (2005). These results could be explained due to the sensitivity of *S. aureus* to heat by employed pasteurization process which support the fact that pasteurization is one of the critical control points that prevents contamination. Also, street vendors' awareness about handling of cheese is weak.

Total coliform counts were carried out to detect fecal contamination. Nearly similar results were reported by Araujo et al. (2002) and Al-Tahiri (2005). The contamination may occur in cheese made by farmers due to using water from wells not under health control, or/and because the chlorination processes are not well implemented for washing and cleaning the utensils which contact milk and cheese. This indicates the decline of individual awareness and health conditions. Higher incidences of *E. coli* in cheese were also reported by Gonzalez et al. (2000) and Araujo et al. (2002). Presence of *E. coli* in the examined samples is an indicator of unsanitary handling and processing and the contamination could be direct from handlers to milk or indirect from the cow's feces as *E. coli* may occur cross contamination.

Salmonella spp. was isolated from four samples (3.5%) of 114 traditional cheeses marketed without refrigeration, agrees with the findings of ICMSF (1998). *Salmonella*, may grow during cheese manufacture. The contamination may have come from the birds and poultry which have been seen indoor and nearby of the manufacturing room. This finding supports that pasteurization process is one of the major critical control points in the cheese-making process that prevents pathogenic.

The reduction of health conditions in manufacturing places and lack of awareness of cheese makers are very important reasons for contamination.

5. CONCLUSION

Listeria monocytogenes was not detected in any of the tested samples. Cheese made in factories has high microbial quality, less or free from pathogenic microorganisms compared with traditional soft cheese. Traditional cheese has high contamination percent especially in coliform group which indicates poor hygienic practice by farmers. The total health risk to the consumer is less from cheese made from properly pasteurized milk than from cheese of similar composition made from unpasteurized milk. The detection of *S. aureus*, *E. coli* and *Salmonella spp.* suggests that soft cheese commercialized in Gaza city may represent a health risk for the consumers.

6. REFERENCES

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