

Incidence of *Salmonella* in Traditional White Cheese in Gaza Markets, Palestine

Iyad A. M. Alzaeem

University College of Science & Technology,
(Gaza, Palestine)
Email: i.zaeem [AT] ucst.edu.ps

ABSTRACT— *Introduction: Salmonella has gained more interest because it cause food borne illnesses. Traditional soft cheese made from unpasteurized milk may contain salmonella.*

Methods: A descriptive study was performed, one hundred and four samples of fresh white cheese collected from street vendors and the local markets to investigate the incidence of salmonella in fresh white cheese in Gaza.

Results: Salmonella was found in four samples of "Traditional cheese". Differences between percentages of samples were not statistically significant.

Conclusion: traditional cheese highly contaminated and usually exceeded the Palestinian Standard. The detection of Salmonella spp. suggests that traditional soft cheese commercialized in Gaza may represent a health risk for the consumers.

Keywords—Traditional white cheese, Salmonella, Food microbiology, Palestine.

1. INTRODUCTION

Cheese classification is based on different criteria such as moisture content into hard cheese (30- 40 %), semi-soft (40-50%) and soft (50-70%) (Abu El-Naja, 2003). It is also classified according to the source of milk (ewes and cows) and according to fat content (Palestinian Standard PS, 1999). 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, the moisture content is no more than 60 %, fat content is never less than 16%, salt concentration ranges from 2 to 4% (increased percentage is allowed if it is written on label). It has a high moisture value and nearly low acidity; this makes the cheese an adequate culture medium for the growth of a wide variety of bacteria. Some of these bacteria are known to cause food-borne illness. 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 (PS). Also, a study made in Agriculture Research Center, Giza, Egypt (2003) showed that, about 90% of 1750 samples of fresh white cheese were contaminated by harmful bacteria. Raw or pasteurized milk and cheese frequently implicated as vehicles of transmission of pathogenic bacteria and with outbreaks all over the world (Flowers et al., 1992). However, the Food and Drug Administration FDA (2005) advises that some soft cheeses made from raw milk present a health risk, especially to high-risk groups such as the pregnant, the newborns, the elderly, and people with weakened immune system. Rampling, likewise, (1996) detected that some outbreaks of food-borne illness has clearly linked with the consumption of cheese. Cheese generally considered a relatively 'safe' food because the pasteurization process of (71.7°C for 15 sec) high-temperature, short time (HTST) is one of the major critical control points in the cheese-making process that prevents pathogenic microorganisms in the raw milk from contaminating the finished product. Although heat treatment destroys some species of microorganisms, some pathogenic bacteria such as *Salmonella* and *E. coli* O157 may survive and thus contaminate the final product even though the milk has received a moderate heat process. Also, recontamination is possible due to insufficient cleaning and disinfection of the exterior of the udder or due to microbial penetration of the ducts (Smith, 1998). The environmental conditions, which have effects on contamination such as dust from sweepings, silage, manure and bedding materials, may contribute to the microbial load found in/ on the udder of the cow. Thus, the number of bacteria in aseptically tired milk from the healthy cows ranges from few hundreds to few thousand organisms per milliliter, other than bacteria, which are rarely found due to infection, disease, or poor milking practices (Harmon, 1995). This Microbiota (microflora) can reach the milk from the udder and may be increased by the labors, tools and handlers during the cheese process, but on the other hand, there are many steps in the process that may decrease the number as well as the types of microbiota such as milk clarification and pasteurization .

Cheese contaminants during manufacturing process

Contamination during manufacturing, handling process, equipment, and temperature abuse, and transport and storage conditions might give high levels of pathogenic microorganisms in cheese. Related to IFST (1998) contaminated cheese has been responsible for outbreaks of food poisoning by several types of bacteria and sporadic cases of illness. Some of these bacteria can cause severe illness with long-term consequences and death. 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.

Salmonella spp.

Salmonella species are small rod shaped, gram-negative, cause infective food poisoning and live in the intestinal tracts of humans and animals, including birds and usually transmitted to humans by eating foods contaminated with animal feces by insects (Trickett, 1997). Contaminated foods usually look and smell normal. It can cause severe illness to infants, the elderly and those with impaired immune systems (CDFA, 2002). *Salmonella* can grow on a temperature ranging between 7°C and 47°C with an optimum 37 °C, pH range from 4.1 to 9.0 with an optimum 6.5-7.5 and NaCl from Nil to 8% (Shehata, 1999). That means the unpasteurized milk is suitable media to growth. Some *Salmonella* spp. can cause septicemia and may result in long-term illness and undulant fever, a severe condition that can be long-lasting and weakening; people get infected if they do not wash their hands after contact with their feces especially those with diarrhea (Trickett, 1997). Although analysis of commercially made cheese rarely results in isolation of *Salmonella*, this organism may grow during cheese manufacture (ICMSF, 1998). Also, *Salmonella* are not very resistant to heat, where they are destroyed in one or two minutes in boiling water.

Cheeses made from unpasteurized milk appear to have been involved in the majority of the reported outbreaks. Many authors (Flowers et al. 1992; Johnson et al. 1990 a.b.c; Kerr et al. 1996; Rampling, 1996; and Zottola & Smith, 1991 and 1993) reported the results; they 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*, and *E. coli* contamination (FDA, 1998).

Stabel (2003) found that food poisoning cases occurred from the consumption of cheeses that were contaminated with *E. coli*, and *Salmonella*. Further outbreaks of food poisoning have occurred through the consumption of contaminated cheeses or pasteurized milk as well as post-pasteurization contamination of dairy products with *Salmonella*, and *E. coli* (Altekruse et al., 1998). Per 100 000 people were taken into account for pathogens *Salmonella enterica*, *Enteropathogenic E. coli*, WHO (2022) consider that, it found that as capacity scores rise, there is a trend for the incidence of foodborne diarrheal illness to decrease. *Salmonella* risk considered a high-risk organism involved in disease outbreaks due to the consumption of contaminated cheese (Altekruse et al., 1998 and Johnson et al., 1990). Salmonellosis is an infection with *Salmonella* that affects all age groups, at greatest risk for severe or complicated disease including infants, the elderly, and persons with compromised immune systems. Unpasteurized cheeses have been implicated in multiple outbreaks and their consumption poses an important health threat, especially among infants, pregnant women, and immune suppressed persons (Jay, 1996).

Raszl et al. (2001) reported that most outbreaks of Salmonellosis are due to raw foods of animal origin or carelessness in food preparation according to Trickett (1997) who believes that the most usual reason is cross contamination. Nevertheless, *Salmonella* food poisoning results from the ingestion of foods containing suitable strains of this genus in significant numbers (Jay, 1996). Half of Salmonellosis cases are caused by two serotypes *Salmonella typhimurium* (ST); and *Salmonella enteritidis* (SE), the soft cheese made with unpasteurized milk is an important vehicle for ST (Villar et al., 1999).

However, the incubation period for *Salmonella* food poisoning are usually between 12 and 24 hours and the main symptoms are diarrhea, abdominal pain, fever, headache and sometime vomiting. Recovery usually takes any period from a day to one week, but the illness can be fatal in the elderly, the sick or the very young (Trickett, 1997). According to the report made by CDC (2005,) approximately 600 persons die each year with acute Salmonellosis, children are the most likely to get Salmonellosis, young children, elderly and the impaired immune system are more likely to have severe illness. In middle east, the fresh white cheese is produced from pasteurized milk especially in licensed factories but most cheese production in farm or house (traditional) comes from raw milk (i.e. milk that has received few heat-treatment). The objectives of this work to study the incidence of *Salmonella* spp. in fresh white cheese in terms of Total Bacterial Count (T.B.C) as indicator of sanitary quality of cheese samples commercialized in Gaza.

2. METHODOLOGY

The boundary of the research is Gaza Strip.

2.1 Cheese Samples

Hundred and four of traditional cheese samples were collected from street vendors and some retailers in the main five markets in Gaza were processed on the day of acquisition. The samples immediately transported in plastic icebox to the laboratory in retail packages. All samples were analyzed within their assigned shelf lives, and the storage temperature at local retail markets in the Primary Health Laboratory (PHL) in Gaza City in retail packages. Microbiological analyses 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).

2.2 Compliance of results and Data analysis

According to PS 22/1997, the soft cheese must be free from foreign matter and spoilage and free of pathogenic bacteria. The results of this work compared consideration the microbiological criteria of the sample that was taken from the market, which are the maximum limits of coliform is 2×10^3 (CFU/g). Hence, the increase of these limit defined as non-complied or not accepted results in this paper. Data entered and tabulated by using Microsoft Excel and SPSS (Statistical Package for the Social Sciences). P value less than 0.05 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.

2.3 Microbiological Determinations

2.3.1 Total Bacterial count

Procedures for detection of T.B.C tested according to the method of FDA (1995). Serial dilutions of soft cheese samples (10^{-2} - 10^{-6}) were prepared; one ml of each dilution was streaked under aseptic condition onto the surface of nutrient agar medium and incubated at 37 °C for 24 hr.

2.3.2 Detection and isolation of Salmonella spp.

Detection and isolation of *Salmonella* spp. were followed according to the method of FDA (1995). The following steps were applied in order to complete confirmation: Twenty five gram of soft cheese were added to 225 ml of lactose broth, then mixed and homogenized. The mixture was transferred under aseptic condition to 500-ml. jar, let stand for 60 min at room temperature and incubate at 35 °C for 24 hour. For enrichment, 2 ml of pre-enrichment mixture transferred to 10 ml Selenite Cysteine broth (SCB) and Tetrathionate Broth (TTB) (one ml. for each broth) and incubated for 24 ± 2 hr at 35°C. For sub culturing, a loop of enriched culture was streaked onto Bismuth Sulphite Agar (BSA), Xylose-Lysine Desoxycholate Agar (XLDA) and *Salmonella-Shigella* Agar (SSA) and incubated for 24 ± 2 hr at 35°C. Suspect colonies from each medium were chosen: For (BSA) medium, brown, gray, or black colonies. For (XLDA) medium, pink colonies with black colour in centres. For (SSA) medium, black colonies. Two or more typical colonies were inoculated onto slants of Triple Sugar Iron Agar (TSIA) and Lysine Iron Agar (LIA) media and incubated for 24 ± 2 hr at 35°C. Suspect colonies picked to perform serological tests.

3. RESULTS AND DISCUSSION

3.1 Total Bacterial Count (T.B.C.)

T.B.C is a general indicator of microbial contamination of cheese. Table 1 shows that the total count in traditional soft cheese is ranged between Nil and 3×10^6 (CFU /g) and presented mean value 3.2×10^5 . Meanwhile, Aly & 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) and AL-Tahiri, (2005) found 2×10^4 in cheeses produced by farmers. However, Aly & Galal (2002) and AL-Tahiri, (2005) reported lower counts of T.B.C (5×10^3 and 50 CFU /g) respectively. On the other hand, soft cheese produced by some farmers, and marketed under refrigeration conditions containing bacterial count with mean values 1.3×10^5 less than the other produce by farmers and marketed without refrigeration, which had a total bacterial count ranged between Nil and 3×10^6 , with a mean value of 3.3×10^5 . The increase of T.B.C can explained by the environmental conditions that allow the growth and multiplication of microorganisms. This finding agreement with Aly & Galal (2002) that heating who recorded that pasteurized milk cheese demonstrated significant decrease in total count more than raw and heat-treated milk cheese.

Table 1: T.B.C in cheese samples (CFU/g)

Producer	No. of samples	Min	Max	Mean	Std. deviation
Farmers	114	Nil	3×10^6	3.2×10^5	4.5×10^5
Marketing method					
Refrigeration	57	Nil	1×10^6	1.3×10^5	2.3×10^5
Without refrigeration	57	Nil	3×10^6	3.3×10^5	4.8×10^5

Table 2 shows that the highest T.B.C frequency distribution lies within the range 10^5 to $< 10^6$ (CFU/g cheese) that reached 50% in traditional soft cheese samples and only one sample (0.9%) less than 10^3 (CFU /g).

Table 2: Frequency distribution of examined samples based on T.B.C (CFU/g)

Range of counts (CFU/g)	No. of samples	Percentage
Nil	1	0.9
10^2 - $<10^3$	0	0
10^3 - $<10^4$	17	14.9
10^4 - $<10^5$	36	31.6
10^5 - $<10^6$	57	50
$10^6 \leq 10^7$	3	2.6
Total	114	100

3.2 *Salmonella* spp.

Results in Table 3 shows that *Salmonella* spp. was isolated from four samples (3.5%) of 114 traditional cheeses made by farmers. The detection of *Salmonella* spp. may represent a health risk for the consumers according to Johnson et al. (1990). However, *Salmonella* is not very resistant to heat that means the absence of *Salmonella* in the other samples maybe due to the heat treatment process or due to the addition of preservatives, which prevents the growth of *Salmonella* spp. as reported by Kasrazadeh and Genigeorgis (1995). On the other hand, from these four samples *Salmonella* ssp. was isolated from three samples (5.3%) of 57 traditional cheeses marketed without refrigeration, while the organism was isolated from one sample (1.7%) of 57 cheese samples marketed in refrigeration. However, the difference between two groups did not arrive at statistical significant ($P=0.26$). 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 cheese contamination. This result agrees with the findings of ICMSF (1998). However, the variation between two groups did not arrive at statistical significant ($P = 0.95$). Thus, soft cheese has been thoroughly pasteurized and is served within a short time will not be a cause for *Salmonella* food poisoning.

The mean value for T.B.C was 3.2×10^5 (CFU/g cheese). The high moisture content with mean 63 % and nearly natural pH (6–7), and the low content of salt with mean value of 2.65%. These conditions of chemical characteristics add to the microbial contamination encourage and permit the growth and multiplicity of microorganisms in soft cheese. This comes in agreement with Aly & Galal (2002).

Table 3: Distribution of *Salmonella* spp. in cheese samples made by farmers according to marketing method

Producer	No. of samples	Accepted (Neg) No. (%)	Not accepted (Pos.) No. (%)	Odds Ratio		P. value
				Value	95% C.I	
Farmers	114	110 (96.5%)	4 (3.5%)	-	-	-
Marketing method						
Refrigeration	57	56 (98.24 %)	1 (1.75%)	0.29	0.03-2.9	0.26
Without refrigeration	57	54 (94.7%)	3 (5.3%)			

C.I: Confidence Interval.-: Odds ratio not calculated

The high numbers of microorganisms in traditional cheese are indicators of contamination by direct contact of milk with unclean surfaces such as those of milking utensils and the hands of laborers besides environmental factors such as uncleanliness of manufacturing place and its surroundings.

3.3 Comparison between cheese results based on marketing method

Table 4 summarizes the results of the cheese samples. The contamination levels were higher in the traditional cheese made by farmers and sold by street vendors without refrigeration than those sold by retailers and preserved in refrigeration. The T.B.C was $>10^5$ CFU/g in 24 samples of 57 (27.6%) marketed in refrigeration, while it was 45 out of 57 (51.7%) for samples of traditional cheese marketed without refrigeration respectively. and the difference was a high statistically significant ($P=0.001$).

Results detect four samples positive of *Salmonella* spp. One sample detected positive of 57(1.1%) traditional cheese produced by farmers and marketed in refrigeration; whereas, the other three samples detected positive (3.4%) were

marketed without refrigeration and the association did not reach statistical significant (P=0.26).

Table 4: Comparison of microbial contamination based on marketing method

Microorganisms	Marketing method				P. value
	Refrigeration		Without refrigeration		
	No. of samples	Not accepted No. (%)	No. of samples	Not accepted No. (%)	
T.B.C	57	24 (42.1%)	57	45 (79%)	0.001*
<i>Salmonella spp.</i>	57	1 (1.75%)	57	3 (5.3%)	0.26

*: statistically significant

4. CONCLUSION

Traditional cheese has high contamination percent especially in T.B.C, which indicates poor hygienic practice by farmers. Samples collected from farmers in Gaza had higher levels of contamination. 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.

5. RECOMMENDATION

Concerned authorities should give much attention and control; there is a definite need for knowledge and continued educational programs to increase the awareness of farmers, the safe handling of dairy products. Therefore, the recommendations is imposing and enacting official regulations regarding cheese production and selling places, which include improving healthy environment conditions. All cheese-processing places should have official licenses and be under periodic supervision by the authorities. Increase awareness for the public regarding health risks from homemade cheese.

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