Incidence of Staphylococci with Special Reference to *Staphylococcus aureus* in Two Types of Locally Made Soft Cheese in Tripoli, Libya.

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Abstract

One hundred locally made white soft cheese samples (fifty each of Ricotta "traditional name" and Maasora) were randomly collected from different localities in Tripoli city (Dairy shops and dairy plants). The objectives of this study were: (i)- To clear the incidence rate of staphylococci in locally made soft cheese samples manufactured by traditional methods; (ii)- To correlate the incidence of Staphylococcus aureus as compared with total staphylococcal count & (iii)- To suggest the control measures that could be applied to rise the keeping quality and shelf-life of locally produced soft cheese. Staphylococci was present in 86 & 94% concerning the Ricotta & Maasora cheese respectively, while Staphylococcus aureus was present in 36 & 62% for the same cheese types respectively. The mean counts for the total staphylococci were 73×10^4 & 15×10^6 for the two cheese types respectively, while for Staphylococcus aureus count, the mean counts were 26×10^2 and 37×10^4 for the same two cheese types respectively. The difference in incidence and count between the two cheese types may be related to the difference in manufacture due to the use of heat treatment in Ricotta cheese and not in Maasora cheese. The risk factors of such higher counts and factors that may limit such higher counts in locally manufactures soft cheese were discussed.

Introduction

Food-borne outbreaks due to consumption of dairy products constitute a chronic problem facing food hygienists. Because milk and dairy products are subjected to different sources of contamination by the food poisoning pathogens either from endogenous origin or directly and indirectly from exogenous origin (*15; 8*). The origin of contamination by food poisoning organisms varies with the type of product and the mode of production and processing *(12)*. Treatment and processing of milk inhibits or encourages the multiplication of such organisms. Milk and dairy products

contain all the nutritional components that make milk and milk products an important part of the human diet, on the other side, these nutrients also support the growth of these pathogenic organisms.

Staphylococcus aureus was the third most common cause of confirmed food poisoning in the world till the year 2003 (*1*). Nowadays, it is reported that the entero-toxin of *Staphylococcus aureus* is the second major cause of food poisoning outbreaks *(9)*.

The organism is a common cause of mastitis in dairy cattle, and it can also enter the milk supply from sores on the teats of dairy cattle or from the hands and nasal discharges of dairy farmers and workers.

The invading organisms may find opportunities to grow and multiply in the products producing a potent entero-toxin that induce food- poisoning outbreaks among consumers, especially when foods are held at temperatures above 10°C *(16)*.

The diagnosis of Staphylococcal food-poisoning is generally confirmed by at least one of the following (i)- the recovery of $>10^5$ *S. aureus*/g from the food remnants, (ii) the detection of staphylococcal enterotoxins in food remnants & (iii) the isolation of *S. aureus* of the same phage-type from both the patients and food remnants (*4*).

Realizing that staphylococcus entero-toxins are thermo- stable and not easily destroyed by heat, therefore, such contaminated products can induce the illness even if they are heat-treated *(14)*.

Owing to the continuous demand for the milk and dairy products, it is extremely necessary not only to increase the production of milk and its products but also to safeguard consumers against health hazard.

Therefore, this study was planed to evaluate the microbial status of the locally produced white soft cheese by counting, isolation, and identification of staphylococci.

Materials and Methods

One hundred white soft cheese samples (Fifty each of Ricotta and Maasora) were randomly collected from different localities in Tripoli city. Collected samples were transferred to the laboratory to be immediately examined for total staphylococcal count as well as *S. aureus* count.

Collection of Samples

Samples were collected from groceries, small dairies and dairy plants in sterile airtight sampling bags. The obtained samples were kept at temperature of 4°C and were analyzed within 24 hours of collection as recommended by (*7*).

Preparation of the collected samples

The collected samples of each product were sent to the laboratory to be prepared according to American Public Health Association *(2)*. Twenty five grams of each cheese sample (Ricotta and Maasora) were weighed and added to 225 ml of sterile 2% sodium citrate solution in polyethylene bags and blended for 2 minutes to prepare dilution 1/10.

(a)- Manufacture of Ricotta cheese:

The raw milk is boiled and NaCl is added in required percent (mostly not more than 2%), then acidified using acetic acid and/or Lemon juice, stirred slowly until curd formation. The obtained curd is pressed gently and transferred to special baskets that gives the shape of the cheese and left for a time then kept in refrigerator until its marketing (*5*). This type of cheese has no relation with internationally known Ricotta cheese made from whey protein.

(b)- Manufacture of Maasora cheese:

The raw milk is just warmed at 37 to 40°C to be suitable for rennet addition, the rennet is added with continuous stirring and left until curd formation, the curd is strained and pressed in special baskets until obtaining the required texture, kept in refrigerator until marketing. This

type of cheese contains lower moisture percent than that of local Ricotta cheese (*5*).

Experimental procedure:

Enumeration of Staphylococci (3):

For each dilution to be plated, aseptically transfer 0.1 ml sample suspension to 3 plates of Baird-Parker agar medium. The inoculum was distributed over surface of agar plates using sterile bent glass streaking rod. Plates were inverted and incubate at 35°C for 48 hours. Suspected colonies [circular, smooth, convex, moist, 2-3 mm in diameter on un-crowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle]. In plates with 20-200 colonies, were counted and recorded.

Staphylococcus aureus count and isolation:

Staphylococcus aureus presumptive count was made using Baird-Parker agar according to (*11*). Pure separate suspected colonies of *S. aureus* (Metallic black colonies, surrounded by double zone) were picked up from Baird-Parker plates and transfer to nutrient agar slants and incubated at 37°C for 24 hours. Purified isolates will subject to different biochemical reaction (coagulase test, anaerobic utilization of glucose, thermostable nuclease production as well as mannitol fermentation) according to (*2*).

Results and Discussion

Table 1: Incidence of staphylococci and Staphylococcus aureus in examined cheese samples.

Cheese type	Total Staphylococci		Staph. aureus	
	No	%	No.	%
Ricotta (50 samples)	43	86	18	36
Maasora (50 samples)	47	94	31	62

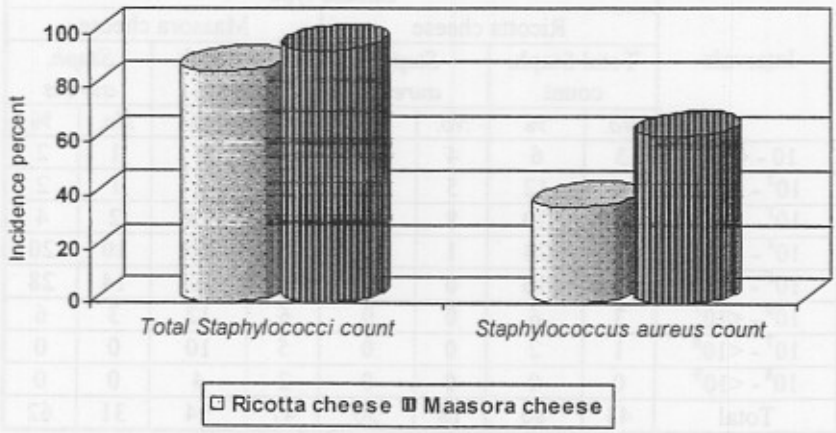

Figure 1: Incidence of staphylococci and Staphylococcus aureus in examined cheese samples.

Table 2: The mean count "CFU/g) for positive cheese samples concerning total taphylococci and Staphylococcus aureus counts.

Count		*Minimum*	*Maximum*	*Mean ± SE*
Ricotta cheese	*Total staph.*	*10*	43×10^{6}	$73\times10^{4}\pm13\times10^{4}$
	Staph. aureus	*10*	81×10^{3}	$26\times10^{2}\pm7\times10^{2}$
Maasora cheese	*Total staph.*	*10*	75×10^{7}	$15\times10^{6}\pm2\times10^{6}$
	Staph. aureus	*10*	9×10^{6}	$37\times10^{4}\pm6\times10^{4}$

Table (3). Frequency distribution of total staphylococci and *Staphylococcus aureus* counts in examined locally made soft cheese.

Intervals	Cheese type							
	Ricotta cheese				Maasora cheese			
	Total Staph. count		*Staph. aureus*		Total Staph. count		*Staph. aureus*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
10 - $<10^{2}$	3	6	4	8	1	2	1	2
10^{2} - $<10^{3}$	6	12	5	10	3	6	1	2
10^{3} - $<10^{4}$	10	20	8	16	9	18	2	4
10^{4} - $<10^{5}$	12	24	1	2	10	20	10	20
10^{5} - $<10^{6}$	8	16	0	0	11	22	14	28
10^{6} - $<10^{7}$	3	6	0	0	6	12	3	6
10^{7} - $<10^{8}$	1	2	0	0	5	10	0	0
10^{8} - $<10^{9}$	0	0	0	0	2	4	0	0
Total	43	86	18	36	47	94	31	62

Staphyococci was present in Ricotta cheese (locally made soft cheese made form boiled milk) in 43 samples (86%), while present in 47 samples (94%) of Maasora cheese (Locally made soft cheese made from raw milk), concerning Staphylococcus aureus, the incidence percent was 36 (18 samples) and 62% (31 samples) concerning the two types of cheese respectively (Table 1 & Fig. 1). These results are in agreement with (6).

Table 2 declares that the mean count of staphylococci was $73\times10^4\pm13\times10^4$ CFU/g in cheese known as Ricotta cheese, the minimum count was 10 CFU/g, while the maximum count was 43×10^6 CFU/g. The Staphylococcus aureus count for the same cheese type was $26\times10^2\pm7\times10^2$ CFU/g with a minimum count of 10 and maximum count of 81×10^3 CFU/g.

The mean count for Maasora cheese concerning total staphylococcus count was $15\times10^6\pm2\times10^6$ CFU/g, with a minimum count of 10 and maximum count of 75×10^7 CFU/g. The Staphylococcus aureus count for Maasora cheese was $37\times10^4\pm6\times10^4$ CFU/g with a minimum count of 10 and maximum count of 9×10^6 CFU/g. These results are in agreement with (6;11).

The higher frequency distribution for total staphylococci concerning Ricotta cheese (24%) was between 10^4 and 10^5 CFU/g, while for Maasora cheese the percent was 22% and lies between 10^6 and 10^7 CFU/g. For Staphylococcus aureus the higher frequency distribution in Ricotta cheese lies between 10^3 and 10^4 and was 16% while for Maasora cheese lies between 10^5 and 10^6 and was 28%, this was demonstrated in table 3.

It is worth mentioning that the highest frequency distribution of S. aureus in Maasora cheese (28%) lies between 10^5 and 10^6 (Table 3), this is because the recovery of $> 10^5$ Staphylococcus aureus from cheese may gives alert for danger for food poisoning with Staphylococcus aureus enterotoxin in case of presence of enterotoxigenic strain (s) of Staphylococcus aureus (9).

The count for total staphylococci as well as Staphylococcus aureus seems to be unacceptable concerning the hygienic point of view generally and the suggested Libyan Standards specifically, this high count in both cheese types may be due to the unhygienic measures during handling, processing and transportation of these types of cheese (6). Other reason for the high count in Ricotta cheese was due to the high moisture percent of this type of cheese (13).

The relatively higher count for both total staphylococcus count and Staphylococcus aureus count in Maasora cheese as compared with

the same counts for Ricotta cheese may be due to using raw milk for Maasora cheese. The comparatively lower moisture percent "lower a_w" of Maasora cheese due to pressing during manufacturing (only a step in Maasora cheese manufacture) is not sufficient for controlling the contamination level of staphylococci.

For controlling of such contamination, it is important to focus the attention upon the quality of the raw milk before processing to ensure the quality of the end product made from it, also, the unsatisfactory conditions of processing, handing and distribution of traditionally made soft cheese in addition to the lack of efficient veterinary supervision upon foods originated from animal origin may explain the obtained result in this work, this is whey it is recommended to have specific standards for Ricotta and Maasora cheeses and other food products in Libya for judging such products and for supporting the food roles, thus it is suggested to set the standards as quickly as possible.

In conclusion, the use of appropriate hygienic procedure, e.g. Hazard Analysis Critical Control Point system (HACCP) during processing should reduce the likelihood of higher microbial count and possibility of associated outbreaks.

References

1. ***Acco, M.; Ferreira, F. S.; Henriques, J. A. P. and Tondo, E. C. (2003).*** Identification of multiple strains of *Staphylococus aureus* colonizing nasal mucosa of food handlers. Food Microbiology, 20: 498-493.
2. ***APHA "American Public Health Association" (1992).*** Compendium of Methods for the Microbiological Examination of Foods. 2nd Ed, APHA, Washington D. C., USA.
3. ***BAM on line "Bacteriological Analytical Manual Online" (2001).*** U.S. Food & Drug Administration Center for Food Safety & Applied Nutrition
4. ***Bryan, F. L.; Guzewich, J.J., Todd, E.C.D., 1997.*** Surveillance of foodborne disease II. Summary and presentation of descriptive data and epidemiologic patterns; their value and limitations. J. Food Prot. 60, 567–578.
5. ***El- Jerbi, A. M. (1998).*** Evaluation of locally made soft cheese concerning its quality and possibility of manufacture from reconstituted milk. Master Thesis of Food Science, Faculty of Agriculture, Libya.

6. ***Garbaj, A. M.; Naas, H. T.; Gammoudi, F. T. and Moawad, A. A. (2007).*** Bacteriological quality of Mozzarella cheese sold in Tripoli Governorate. Bs. Vet. Med. J., 17: 99 – 104.

7. ***HPA (2003).*** Preparation of samples and decimal dilutions. D1. Issued by Standard Unit, Evaluation and Standards Laboratory. Health Protection Agency (HPA). Specialist and Reference Microbiology Division. Issue no.: 23.

8. ***Jablonski, L. M. and Bohach, G. A. (2001).*** *Staphylococcus aureus*, In: Doyle, M. P., Beuchat, L. R., Montvile, T.J. (Eds.), Food Microbiology: Fundamentals and Frontiers, 2nd ed. ASM Press, Washington, D. C., USA, pp. 411- 434.

9. ***Kerouanton, A.; Hennekinne, J. A.; Letertre, C.; Petit, L.; Chesneau, A.; Brisabois, A. and De Byser, M. L. (2007).*** Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France, International Journal of Food Microbiology, 115: 369 - 375.

10. ***Lancette, G. A. and Tatini, S. R., 1993.*** *Staphylococcus aureus* Chapter 33, In Compendium of Methods for the Microbiological Examination of Foods. Inc. 4th Ed., New York, Vanderzantic, C and Splittstoesser, D. (Eds.) American Public Health Association, Washington, DC, USA.

11. ***Lina Casale, A. A.; Eliziani, M. K.; Karina, S.; Márica, G. S.; Ary, F. J. Ricardo, R. and Vera Lùcia, M. R. (2007).*** Occurrence of coagulase-positive staphylococcus in various food products commercialized in Botucatu, SP, Brazil and detection of toxins from food and isolated strains, Food Control, 18: 630 – 634.

12. ***Moawad, A. A. and El- Neary, N. A. (1996).*** *Microbial studies on creamy "Kishda" cheese sold in Egyptian markets. Benha Vet. Med. J., 2: 7.*

13. ***Naas, H. T.; Gammoudi, F. T.; Garbaj, A. M.; Azwai, S. M. and Moawad, A. A. (2007).*** Comparison between two different conventional methods for coliform count in raw milk and locally made soft cheese in Tripoli-Libya. Benha Vet. Med. J., vol.18, No. 1, June. 2007

14. ***Saudi, A. M. (2002).*** Milk, Dairy products, Eggs and Edible Fats & Oils, first Ed. Cairo University Publishing Center.

15. ***Shimizue, A.; Fujita, M.; Igarashi, H.; Takagi, M.; Nagase, N.; Sasaki, A. and Kawano, j. (2000).*** Characterization of *Staphylococcus aureus* coagulase Type VII isolates from staphylococcal food poisoning outbreaks (1980- 1995) in Tokyo, Japan, by pulsed-field gel electrophoresis. J. Clin. Microbiol., 38: 2746- 3749.

16. ***Tsung, C. and Huang, S. U. H. (1993).*** An immuno-Linked immunosorbant assay for rapid detection of *Staphylococcus aureus* in processed food. J. Food Prot. 75: 184.

مدى تواجد الميكروبات العنقودية وبخاصة الميكروب العنقودى الذهبى فى نوعين من الجبن المصنع محلياً فى طرابلس - ليبيا

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تم جمع عدد ١٠٠ عينة بشكل عشوائى الجبن الأبيض الطرى المصنع محلياً وذلك بواقع ٥٠ عينة من الجبن الريكوتا (أسم محلى لجبن مصنع من حليب معامل حرارياً) وأخرى من الجبن المعصورة (جبن مصنع من حليب خام) وذلك من مصانع الجبن الطرى والمتاجر الغذائية وقد كان الهدف من الدراسة تحديد مدى التلوث بالميكروبات العنقودية من خلال تحديد العدد الكلى لهذه الميكروبات وكذلك لتحديد العدد الإفتراضى للميكروب العنقودى الذهبى وكذلك لاقتراح المقاييس المناسبة للتقليل من حد التلوث بتلك الميكروبات والاهتمام بالنواحى الصحية للإنتاج المحلى. وقد تواجدت الميكروبات العنقودية فى ٨٦، ٩٤% من الجبن الريكوتا والمعصورة على التوالى وكان متوسط العدد الكلى هو ٧٣×١٠^{٤} و ١٥×١٠^{٦} على التوالى. أما بالنسبة للميكروب العنقودى الذهبى فقد تواجد بنسبة ٣٦، ٦٢% للجبن الريكوتا والمعصورة على التوالى وذلك بمتوسط عددى ٢٦×١٠^{٢} و ٣٧×١٠^{٤} لكلا النوعين على التوالى،

وقد أتضح من النتائج أن التركيز الأعلى للتوزيع التكرارى (٢٨%) لعينات الجبن المعصورة كان مابين ١٠^{٥} و ١٠^{٦} وهو العدد البكتيرى اللازم من الميكروبات العنقودية الذهبية لإحداث التسمم الغذائى بهذا الميكروب إذا ما توافرت الظروف لذلك (بالنسبة للعترات المنتجة للسم المعوى). ويتضح من الدراسة أن هناك فارق واضح فى التواجد العددى للميكروبات العنقودية فى كلا النوعين من الجبن والذى قد يرجع إلى استخدام المعاملة الحرارية فى حالة الجبن الريكوتا وأستخدام الحليب الخام فى تصنيع الجبن المعصورة. هذا وقد تمت مناقشة دلالة وجود وخطورة مثل هذه الميكروبات وكذلك التوصية بإتباع الاشتراطات الصحية فى التصنيع والتداول والعرض حتى وصول الجبن للمستهلك.