# OCCURRENCE OF *STAPHYLOCOCCUS AUREUS*, *SALMONELLA* SPECIES AND *LISTERIA MONOCYTOGENES* IN FARMS AND MARKETS MILK

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## ABSTRACT

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A total of 200 random samples of milk from dairy shops, farms and markets (100 farm's milk samples from Cairo, 50 milk samples from different dairy shop in Port-Said and 50 samples of Ultra heat Treatment (UHT) milk from different Port-Said city markets) for isolation and identification of *Staph aureus, Salmonella* species and *Listeria monocytogenes*. The results revealed that the *staph aureus* could be detected in 9% and 28% of farm's and dairy shops milk samples, respectively, with count values 3.6 $x10^2$ and $5.2x10^2$, respectively. Out of 14 strains obtained from dairy shops milk, only two were enterotoxigenic belonging to type A&D and C. Nine *Staph aureus* were obtained from farm's milk, one was enterotoxigenic belonging to type A. While, *Salmonella* species and *Listeria monocytogenes* were isolated from farm's milk only in percent of 5% and 4 %, respectively. The isolated *Salmonella* serotypes were *Salmonella anatum* (one isolate) and *Salmonella* typhimurium (four isolates). On the other hand the Ultra Heat Treatment (UHT) milk from different markets were free from *Staph aureus, Salmonella* species and *Listeria monocytogenes*. The public health hazard of these microorganisms as well as recommended measures to improve quality status of milk were discussed.

***Key words:*** *Milk, Farms, Dairy shops, Markets, Ultra heat Treatment (UHT) milk, foodborne disease (Staph auras, Salmonella species and Listeria monocytogenes), Public health.*

## مدي تواجد ميكروب العنقودي الذهبي والسالمونيلا والليستيريا منوسيتوجينز في ألبان المزارع والمحلات

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يعد اللبن من الأغذية الواسعة التداول لدى الكبار والأطفال ، وتلوثه بالميكروبات الضارة من الأمور التي تستوجب الاهتمام والدراسة، من هنا كان الاهتمام بتحديد مدي تواجد كل من الميكروب المكور العنقودي الذهبي، ميكروبات السالمونيلا وأيضا ميكروب الليستيريا منوسيتوجينز لما لهم من خطورة كبيرة علي الصحة العامة للإنسان ، لذلك تضمنت هذه الدراسة فحص ١٠٠ عينة عشوائية من المزارع و ٥٠ عينة من محلات الألبان و ٥٠ عينة من اللبن المعالج بالحرارة من المحلات المختلفة. وأظهرت النتائج تواجد ميكروب المكور العنقودي الذهبي في عينات اللبن من

المزارع والمحلات بالنسب الآتية ٩ %، ٢٨% علي التوالي وكذلك كان العد البكتيري الكلي للميكروب بالنسب 10x3,6 $^2$، 5,2 x 10 $^2$ علي التوالي. وتم تصنيف العترات المعزولة من عينات اللبن من المزارع والمحلات لمدي إفرازها للسموم ولوحظ أن نوع السم A,C,A&D بينما كانت العينات اللبن المعامل حراريا خالية من الميكروب المكور العنقودي الذهبي . وأما بالنسبة لكل من ميكروبات السالمونيلا وميكروب الليستيريا منوسيتوجينز قد تم عزلهما بنسبة 5%، ٤% علي التوالي من عينات اللبن من المزارع فقط. وقد تم تصنيف العترات المعزولة من السالمونيلا إلي عتره "*Salmonella anatum*" وأربع عترات "*Salmonella* Typhimurium". وأظهرت أيضا أن كل عينات اللبن المعالج بالحرارة من المحلات المختلفة خالي من ميكروب المكور العنقودي الذهبي، ميكروبات السالمونيلا وميكروب الليستيريا منوسيتوجينز. هذا وقد تمت مناقشة الأهمية الصحية لهذه الميكروبات ومدى تأثيرها على الصحة العامة للإنسان وكذلك الشروط الواجب توافرها وإتباعها للمحافظة على سلامة اللبن.

## INTRODUCTION

Milk is nutritious food for human which plays an important role in human diet all over the world, but at the same time it is a good medium for the growth of a wide range of micro-organisms especially pathogens. The presence of such organisms in milk represents a major public health concerns (Ryser, 1998). Milk can carry dangerous bacteria such as *Salmonella* species, *Staph aureus* and *Listeria monocytogenes*, which are responsible for causing numerous foodborne illnesses (De Buyser *et al.*, 2001 and Oueslati *et al.*, 2011). Bacteria multiply rapidly in milk due to its rich nutritional composition. In this concern Boycheva *et al.* (2003), studied bacterial quality of milk and found different types of bacteria in milk like *Listeria* species and *Staphylococcus* species. These harmful bacteria can seriously affect the health of anyone who drinks raw milk, or eats foods made from raw milk. However, the bacteria in raw milk can be especially dangerous to pregnant women, children, the elderly, and people with weakened immune systems.

Staphylococcal food poisoning is a common form of foodborne illness, which results from ingestion of toxins produced by toxigenic strains of *Staph aureus*. Enterotoxins are groups of single chain protein (polypeptides) with molecular weight ranging from 28.000 to 35.000 Daltons, resistant to high temperature (heat stable) and proteolytic enzymes. The enterotoxigenic strains of *Staph aureus* produce several types of enterotoxins (A, B, C, D and E) which can cause symptoms of intoxications such as vomiting, diarrhoea and abdominal cramping (Korpysa *et al.*, 2005). *Staph.* Enterotoxin A (SEA) is responsible for majority of staphylococcal food poisoning whereas *Staph* Enterotoxin B (SEB) is rarely involved (Robbins *et al.*, 1974). In addition, most outbreaks as recorded by Halpin-Dohnalek and Marth (1989) resulted from the combined effect of contamination of food with *Staph aureus* often through unsanitary handling, and holding the food at the wrong temperature thus enhance growth and synthesis of enterotoxins. However, the enterotoxication generally is not lethal and the elderly are more susceptible than younger individuals. The concentration of enterotoxin necessary to cause intoxication is very small about 94-184 ng (Erol and Iseri, 2004).

*Salmonella* species are the most prevalent pathogens in the food industry. Studies about these microorganisms date up to 100 years and have been the causative agent on several outbreaks of foodborne diseases particularly in dairy products. Most of species are pathogenic. The primary habitat of *Salmonella* species is the intestinal tract of animals and humans. Milk is an important vehicle for *Salmonellae* causing human infection. Additionally *Salmonella* species causes illness by means of infection. They multiply in the small intestine, colonizing and subsequently invading the intestinal tissues, producing an enterotoxin and causing an inflammatory reaction and diarrhea (ICMSF, 2006).

*Listeria* species, cause the infection of listeriosis in both animals and man, *Listeria monocytogenes* is a major pathogenic microorganism (Aygun and Pehlivanlar, 2006). Listeriosis caused by *Listeria monocytogenes* has increased drastically in

recent years (Choi and Hong, 2003). *Listeria monocytogenes* associated with septicemia, meningoencephalitis and abortion in humans and animals, primarily affecting pregnant, new-born, and immuno-compromised individuals (Choi and Hong, 2003; Rossmanith *et al.*, 2006, Mugampoza *et al.*, 2011). Several outbreaks of listeriosis were proven to be associated with the consumption of milk and are causing great concern in the dairy industry due to high mortality rate (nearly 30%) of these outbreaks (Amagliani *et al.*, 2004). *Listeria monocytogenes* can gain entry to the milk from faecal contamination of the udder. Once introduced into the milking equipment, *Listeria monocytogenes* can readily colonize these moist environments. *Listeria monocytogenes* is found commonly in wet areas of dairy plants, such as floor drains, conveyers, floors and stainless steel equipment (Bell and Kyriakides, 2002).

Contamination of milk and dairy products by pathogenic micro-organisms can be of endogenous origin, following excretion from the udder of an infected animal or may be also of exogenous origin, through direct contact with infected herds or through the environment (e.g. water and personnel, etc.). Bacteria most frequently involved are *Listeria monocytogenes, Staphylococcus aureus* and enterobacteria (including *Salmonella*) (Brisabois *et al.*, 1997) and (Ben Hassen *et al.*, 2003). High microbial counts in milk usually result in inferior quality of milk (Costello *et al.*, 2003). Adulteration of milk, affect also compositional and microbiological quality of milk. In Egypt; climatic conditions; low level of sanitation during production; transportation and handling are responsible for low quality of milk and dairy products (Girgis *et al.*, 1996).

This study is planned to investigate the presence of *Staph aureus, Salmonella* species and *Listeria monocytogenes* in milk with regarding to the public health.

## MATERIALS and METHODS

**1- Samples collection:**

A total of 200 random samples of milk were collected from different farms in Cairo, different dairy shops and different markets in Port-Said city (100 samples of farm's milk from Cairo, samples of dairy shops from Port-Said 50 city and 50 samples of Ultra Heat Treatment (UHT) milk from markets in different localities in Port-said city for detection of *Staph aureus, Salmonella* species and *Listeria monocytogenes*. The samples were collected in clean, dry and sterile containers. Collected samples were transferred in an ice box and transported to the laboratory as soon as possible to be examined.

**2- Bacteriological examination:**

**2-1 *Staphylococcus aureus*:**

Twenty-five ml of each dairy shops, farm's and Ultra Heat Treatment (UHT) milk samples were homogenized in a stomacher for 2 min in 225 ml of Buffered peptone water, and *Staphylococcus aureus* was enumerated on Baird-Parker with Rabbit Plasma Fibrinogen (BP+RPF, Oxoid) after incubation for 48 h at 37 °C ISO, 6888-2(1999).

**2-1-1 Detection and typing of Enterotoxins from *Staphylococcus aureus*:** according to the method recommended by (Oda *et al.*, 1979 and Shingaki *et al.*, 1981). Passive Latex agglutination technique using Oxoid SET-RPLA [A kit for the detection of Staphylococcal enterotoxins A, B, C and D].

**2- 2 *Salmonella* species:** Twenty-five ml of each dairy shops, farm's and Ultra Heat Treatment (UHT) milk samples were homogenized in a stomacher for 2 min in 225 ml of Buffered peptone water, and incubated at 37°c ± 1°c for 18±2 hr. Isolation and identification according to the method recommended by ISO, 6579 (2002).

**2-3 *Listeria monocytogenes*:** Twenty-five ml of each dairy shops, farm's and Ultra Heat Treatment (UHT) milk samples were homogenized in a stomacher for 2 min in 225 ml of *Listeria* enrichment broth (Difco), and incubated at 30°c for 48 hr. Isolation and identification according to the method recommended by ISO, 11290-1 (2011).

## RESULTS

**Table 1:** Incidence of *Staph aureus*, *Salmonella* species and *Listeria monocytogenes* in examined samples of milk from farms, dairy shops and markets.

| Location | Examined samples | No. of examined samples | Isolated strains | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Staph aureus* | | *Salmonella* species | | *Listeria monocytogenes* | |
| | | | No. | % | No. | % | No. | % |
| Cairo | Farm's milk | 100 | 9 | 9% | 5 | 5% | 4 | 4% |
| Port-Said | Milk from Dairy shops | 50 | 14 | 28% | 0 | 0% | 0 | 0% |
| Port-Said | Ultra heat treatment milk from markets | 50 | 0 | 0% | 0 | 0% | 0 | 0% |

**Table 2:** *Staph aureus* count / ml of the positive milk samples from farms, dairy shops and markets.

| Examined samples | Minimum | Maximum | Average |
|---|---|---|---|
| Farm's milk | $2 \times 10^2$ | $5 \times 10^2$ | $3.6 \times 10^2$ |
| Milk from Dairy shops | $1 \times 10^2$ | $6 \times 10^2$ | $5.2 \times 10^2$ |
| Ultra heat treatment milk from markets | 0 | 0 | 0 |

**Table 3:** Typing of *Staphylococcus aureus* strains enterotoxins isolated from the examined milk from farms dairy shops and markets.

| Type of examined samples | No. of strains tested | Enterotoxigenic strains | | Type of Enterotoxins | | |
|---|---|---|---|---|---|---|
| | | No. | % | A | C | A &D |
| Farm's milk | 9 | 1 | 11.1% | 1 | - | - |
| Milk from Dairy shops | 14 | 2 | 14.2% | - | 1 | 1 |
| Ultra heat treatment milk from markets | 0 | 0 | 0 | - | - | - |

**Table 4:** Antigenic structure of different *Salmonellae* isolated from the examined farm's milk.

| *Salmonella* serovars | Sero-group | Antigenic structure | | |
|---|---|---|---|---|
| | | [O] | [H] | |
| | | | Phase (1) | Phase(2) |
| *Salmonella* Typhimurium | B | 1,4,[5],12 | i | 1,2 |
| *Salmonella anatum* | E1 | 3,10 [15] [15,34] | e,h | 1,6 |

**Table 5:** *Salmonella* serotypes isolated from the examined farm's milk.

| Serotypes | No | % |
|---|---|---|
| *Salmonella* typhimurium | 4 | 4% |
| *Salmonella anatum* | 1 | 1% |

**Table 6:** Distribution of *Listeria monocytogenes* in positive samples of farm's milk.

| *Listeria* species | Farm's milk | |
|---|---|---|
| | No | % |
| *Listeria monocytogenes* | 4 | 4% |

## DISCUSSION

Milk contaminated with disease-causing bacteria does not smell or look any different from non-contaminated milk, and there is no obvious way for the consumer to know if the milk is contaminated (Julia, 2010). Pathogenic bacteria in milk has been a major factor for public health concern since the early days of the dairy industry. Many diseases are transmissible via milk products. Traditionally raw or unpasteurized milk has been a major vehicle for transmission of pathogens (Vasavada, 1988). The health of dairy herd, milking conditions are basic determinant of milk quality. Another source of contamination by microorganisms is unclean teats. The use of unclean milking and transport equipment contributed also to the poor hygienic quality (Bonfoh *et al.*, 2003).

As seen from Table (1). The incidence of *Staph aureus* was isolated from 9 (9%) farm's milk and 14 (28%) in milk from dairy shops, These results were nearly similar to those reported by Abdel-Hameed *et al.* (2004) who isolated *Staph aureus* from raw milk samples in proportion of 14.38%. However, the current results were less than those recorded by Chye *et al.* (2004), Letiția *et al.* (2011) and Ekici *et al.* (2004) they showed that *Staph aureus* was isolated from more than 60%, 70% and 75% of the raw milk samples and higher than those found by Abdel-Hameed (2006). Tondo *et al.* (2000) reported that 35.2% of food handlers were asymptomatic carriers of *Staphylococcus aureus*, and that 90.4% of raw milk samples. While *Salmonella* species were isolated from farm's milk only in percentage of 5% the results were higher than those recorded by De Reu *et al.* (2004) and Abd El-Atty and Meshref (2007) who couldn't detected *Salmonella* species in raw milk samples.

*Listeria monocytogenes* were isolated from farm's milk only in percentage of 4%. The above mentioned results were higher than those reported by Jensen *et al.* (1996) who demonstrated *Listeria monocytogenes* in only 1.2% of milk samples. However, the results were less than those recorded by De Reu *et al.* (2004) who could isolate *Listeria monocytogenes* in percentage of 6.3%. Poor hygiene often arises from poor handling at the farm, at collection centers, during transportation and at retail points. Common sources of bacterial contamination, especially coliforms, are faeces (of animal or human origin), personnel, water and containers. A high bacterial count reduces the shelf life of milk and enhances the risk of milk-borne bacterial infections and intoxications if the milk is not properly heated or if thermal injured pathogens recover under suitable temperatures (Kayihura *et al.*, 1987).

Coagulase - positive *Staphylococci* species enterotoxin-producing staphylococcal species, *Staphylococcus aureus* in particular, are the leading cause of food-borne illness throughout the world. Sickness results from the ingestion of one or more preformed staphylococcal enterotoxins in staphylococcus contaminated food. The pathogenicity of *Staphylococcus aureus* has been recognized for many years, it may cause mastitis and/or skin diseases in milk-producing animals or lead to 36type of food-borne intoxications in consumers of milk and milk products (Bolstridge and Roth, 1985). Contamination with coagulase-positive *Staphylococci* was particularly high in raw milk from different farms and Markets (above $5x10^2$ cfu/ml). The source of this contamination is difficult to trace due to the ubiquitous nature of staphylococci. *Staph aureus* is carried in the nose of some 30% of persons, who also tend to be skin carriers, and it is frequent in a number of animals (Olsvic *et al.*, 1982). The enterotoxins of *Staphylococci* are remarkably resistant to heat. Baird-Parker (1990) states the temperature conditions for destruction of *Staphylococcus aureus* to be: 0.43 – 8 minutes at 60°C compared to 3 – 8 minutes at 121°C for enterotoxin.

In this study, the prevalence of *Listeria. monocytogenes* (4%) was found in farm's milk. Similar frequency findings of *Listeria monocytogenes* (0-5%) raw milk samples have been reported from different countries such as Austria 1.5% (Deutz *et al.*, 1999), Spain 3.6% (Gaya *et al.*, 1998), India 1.7% (Adesiyun *et al.*, 1996), USA 4.1%

(Rohrbach *et al.*, 1992), Canada 1.9% (Fedio and Jackson, 1990) and Iran 1.6% (Moshtaghi and Mohammadpour, 2007). The disparate levels of contamination which have been reported from localized studies might have been due to variations in regions or to variations in sampling and detection techniques.

Food-borne disease outbreaks associated with *Salmonella* have been known for a long time and continue to be a problem in both developed and developing countries (Bean *et al.*, 1990). Most outbreaks have implicated foods containing eggs or poultry products. Nevertheless, there have been several outbreaks of salmonellosis for which milk or milk products were responsible. Contamination of milk usually takes place by *Salmonellae* from external sources. Sources can be faeces, the farmer or his family, polluted water, dust etc. Healthy cows can also regularly excrete *Salmonellae* in their dung. Salmonellosis is caused by the ingestion of living bacteria of the *Salmonella* group. In contrast to staphylococcal food poisoning, the ingestion of viable cells is necessary for salmonellosis. The number of cells which have to be ingested to cause disease varies according to the type of strain, the type of food consumed and the consumer. Numbers varying from one cell of *Salmonella typhi* to several millions of, for example *Salmonella derby* or *Salmonella anatum*, are mentioned (D'Aoust, 1989). Infants as well as very young and aged people are especially sensitive and a smaller dose can result in disease. In the present study, *Salmonellae* were isolated from farm's milk in a percentage of 5%. None of the milk form dairy shops and ultra heated milk from markets contained *Salmonella* species. *Salmonellae* are sensitive to heat treatment and are readily destroyed at milk pasteurization temperatures. Pathogenic bacteria in milk has been a major factor for public health concern since the early days of the dairy industry. Many diseases are transmissible via milk products. The health of dairy herd, milking conditions are basic determinant of milk quality. Another source of contamination by microorganisms is unclean teats. The use of unclean milking and transport equipment contributed also to the poor hygienic quality (Bonfoh *et al.*, 2003). The machine-milking may increase the incidence of mammary infections either by a role as vectors of pathogens from infected areas to healthy neighborhoods, either by contamination of the teat force, its role is traumatic for the teat canal, while diminishing effect "barrier" (Boudry, 2005). As a result of the research, the samples of milk examined contained pathogenic microorganisms. This may indicate that analyzed milk can contribute a potential risk for public health in the cases that it is consumed or used in the production of dairy products such as cheese, butter, cream and ice cream without being pasteurized or being subjected to a sufficient heat process.

In this study the Ultra Heat Treatment milk (UHT) from markets was free from *Staph aureus, Salmonella* species and *Listeria monocytogenes*. This result agree with the (Egyptian standard, 2005) and (Riadh, 2005) who mentioned that the UHT milk should be free from pathogenic microorganisms.

## CONCLUSION

The presence of *Staph aureus, Salmonella* species and *Listeria monocytogenes* in farm's milk and milk from dairy shops samples recorded in this study is expected as the produced milk is liable to contaminate from different sources (dust, air, water, equipments, milkers and handlers), moreover, the prevailing of bad handling, poor sanitation of equipments and lake of cooling facilities during transportation. Ultra Heat Treatment milk (UHT) from markets which are free from any pathogens indicated that Ultra Heat Treatment milk (UHT) is fit for human consumption because it gave a real indication for the good hygienic practice during production and handling.

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