

PREVALENCE OF SOME FOOD POISONING BACTERIA ISOLATED FROM MILK AND SOME MILK PRODUCTS IN KAFR EL-SHEIKH GOVERNORATE

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ABSTRACT

A total of two hundred samples (100 milk and 50 each of kareish cheese and ice-cream) were collected from different localities in Kafrelsheikh Governorate for detection of some food poisoning bacteria. The results revealed that S.aureus was detected in 13, 14 and 12% with mean counts of 5.8×10^4 , 11.9×10^4 and 3.3×10^4 . While, E.coli was detected in 11, 14 and 14% with mean counts of 2.8×10^4 , 11×10^4 and 3.6×10^4 in milk, kareish cheese and ice cream samples, respectively. Salmonella spp. was not detected in any of the examined samples. The results concluded that milk and dairy products may at times suitable for supporting the growth of these organisms and subsequently be responsible for food poisoning. The public health significance of the isolated organisms was discussed.

INTRODUCTION

Food poisoning is a common, yet distressing and sometimes life-threatening problem for millions of people throughout the world. People infected with food-borne organisms may be symptom-free or may have symptoms ranging from mild intestinal discomfort to severe dehydration and bloody diarrhea. Depending on the type of infection, people can even die as a result of food poisoning (*Trickett, 1980*).

Milk and dairy products are considered as the most perfect food for human from birth to senility. They are not only having good sensory properties, but also containing all nutrients for the body which can prevent or reduce risks of many nutritional deficiency diseases (*Marshall et al., 2003*). Unfortunately, milk produced under superior hygienic condition yet various microorganisms which may gain access during production and storage may grow and affect the quality and safety of such milk (*Marthand Steele, 2001*).

S.aureus is one of the most important reasons for bacterial food poisoning. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins preformed in the food. From 1991 to 2005, *S. aureus* poisoning cases counted about 17.5% of the total bacterial induced food-poisoning cases in Taiwan (*Chiang et al., 2008*). Milk and milk products are common vehicles of *S.aureus* food poisoning (*Jorgensen et al., 2005*).

E.coli has become recognized as a serious foodborne pathogen and has been associated with numerous outbreaks in the UK, Japan and USA (*Scotter et al., 2000*). *E.coli* includes a variety of different types that range from a virulent commensally strains that are present in the normal intestinal flora to highly virulent strains that cause a variety of severe infections in both humans and animals. The pathogenicity of *E.coli* is considered to be mainly determined by specific virulence factors such as adhesions, invasions, toxins and capsule (*Kaper et al., 2004*).

Salmonella is a bacterial infection that can be passed on to humans from domestic and wild animals, including poultry, pigs, cattle, and pets. Many strains of *Salmonella* are the principal pathogens implicated in human food-borne illnesses. But most often, it is caused by drinking

unpasteurized milk (*Majowicz et al., 2010 and Hendriksen et al., 2011*). They cause illness by means of infection, as they multiply in the small intestine, colonizing and subsequently invading the intestinal tissues, producing an enterotoxin and causing inflammatory reaction and diarrhea (*I. C. M. S. F., 2006*).

As information about distribution of different food poisoning organisms in Kafr EL-Sheikh governorate would help in the design of efficient therapeutic approaches and improvement of control measure. Therefore, the objective of this study was planned to determine the prevalence of some food poisoning organisms in milk and some milk products sold in Kafr EL-Sheikh governorate, Egypt.

MATERIALS AND METHODS

This study was carried out with two hundred samples (100 milk and 50 each of kareish cheese and ice-cream) collected from different localities in Kafr El-sheikh Governorate during January to December 2015. Samples were transferred to the laboratory to be examined bacteriologically.

Preparation of serial dilutions (*A.P.H.A., 1992*):

Raw Milk: 11 ml of each milk sample was added to 99 ml sterile peptone water (1%) then thoroughly mixed to obtain 1/10 dilution, from which 10 fold serial dilutions were prepared.

Ice cream: Samples were kept in thermostatically controlled water bath at 44°C for not more than 15 minutes with periodical shaking till melting before examined. Each sample was then thoroughly mixed using sterile stirrer. 11 ml of each sample was added to 99 ml sterile peptone water (1%) to obtain 1/10 dilution, from which 10 fold serial dilutions were prepared.

Kareish cheese: 11 g of each sample was transferred to sterile mortar, and 99 ml of sterile 0.1 % peptone water was added and mixed to obtain 1/10 dilution, from which 10 fold serial dilutions were prepared.

Enumeration, isolation and identification of *S.aureus* and *E.coli*: From the already prepared serial dilutions, 0.1 ml of each dilution was plated on the surface of Baried Parker agar for *S.aureus* and Eosin Methylene Blue agar (E.M.B) for *E.coli* and evenly distributed. The plates were incubated at 37°C for 48 hours. The shiny black colonies surrounded by halo zone were counted as *S.aureus*; while grayish and shiny colonies were counted as *E.coli* (**Bailly and Scott (1998)**). Bacterial isolates were sub-cultured on slope agar for further identification according to **Quinn et al. (2011)**.

Isolation of Salmonella:

Twenty five g or ml of each prepared sample was aseptically added to 225 ml buffered peptone water, thoroughly mixed and incubated for 24 h. at 37°C. From the resultant pre-enrichment culture, 1 ml was inoculated into sterile tubes containing 10 ml of Rappaport-Vassiliadis broth and incubated at 41.5 °C ± 1°C for 24h ± 3h. before streaking onto Xylose-Lysine Deoxycholate agar (XLD) as recommended by **ISO (2002)**. The plates were incubated aerobically at 37°C for 24-48h. Suspected colonies were described for their morphological characteristic appearance, followed by Gram staining before being transferred into slope agar to be subjected for further identification according to **Quinn et al. (2011)**.

RESULTS

Table (1): Incidence of *S.aureus*, *E. coli* and *Salmonella* in the examined raw milk, kareish cheese and ice cream samples.

Products	No. Of examined samples	Salmonella Positive samples		<i>S. ureus</i> Positive samples		<i>E.coli</i> Positive samples	
		No.	%	No.	%	No.	%
Milk	100	0	0	13	13	11	11
Kareish cheese	50	0	0	7	14	7	14
Ice-cream	50	0	0	6	12	7	14
Total	200	0	0	26	13	25	12.5

Table (2): Statistical analytical results of bacterial counts (cfu/ml or g) of the examined milk, Kareish cheese and ice cream samples.

Products	<i>S.aureus</i>			<i>E.coli</i>		
	Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE
Raw milk	1.1×10^4	1.89×10^5	$5.8 \times 10^4 \pm 4.8 \times 10^3$	1.2×10^4	1.8×10^5	$2.8 \times 10^4 \pm 1.5 \times 10^3$
Kareish cheese	3.6×10^4	2.86×10^5	$11.9 \times 10^4 \pm 1.29 \times 10^4$	1.3×10^4	2.04×10^5	$11 \times 10^4 \pm 11.3 \times 10^3$
Ice-cream	1.2×10^4	6.1×10^4	$3.3 \times 10^4 \pm 2.6 \times 10^3$	1.3×10^4	8.1×10^4	$3.6 \times 10^4 \pm 3.4 \times 10^3$

Table (3): Frequency distribution of the examined samples based on their *S. aureus* count.

Products	Positive samples		<i>S.aureus</i> / ml or g							
	No.	%	< 10^4		$10^4 - < 5 \times 10^4$		$5 \times 10^4 - < 10^5$		$10^5 - < 5 \times 10^5$	
	No.	%●	No.	%●	No.	%●	No.	%●	No.	%●
Raw milk	13	13	0	0	7	53.8	4	30.8	2	15.4
Kareish cheese	7	14	0	0	1	14.2	3	42.9	3	42.9
Ice-cream	6	12	0	0	5	83.3	1	16.7	0	0

●: The percentages were calculated according to the number of positive samples.

Table (4): Frequency distribution of the examined samples based on their *E. coli* count.

Products	Positive samples		<i>E.coli</i> / ml or g							
	No.	%	< 10^4		$10^4 - < 5 \times 10^4$		$5 \times 10^4 - < 10^5$		$10^5 - < 5 \times 10^5$	
	No.	%●	No.	%●	No.	%●	No.	%●	No.	%●
Raw milk	11	11	0	0	4	36.4	4	36.4	3	27.2
Kareish cheese	7	14	0	0	2	28.5	2	28.5	3	43
Ice-cream	7	14	0	0	5	71.4	2	28.6	0	0

●: The percentages were calculated according to the number of positive samples.

DISCUSSION

Naturally, *S.aureus* isolates are inhabitants of mucous epithelia and skin of human, dairy cattle and other mammals (*Chu et al., 2012*), and spread by virtue of milker's hand/milking machines (*Seki et al., 1998*). It is one of the most important reasons for bacterial food poisoning, which results from ingestion of toxins produced by toxigenic strains of *S.aureus*. Enterotoxins are groups of single chain protein, resistant to high temperature and proteolytic enzymes. The enterotoxigenic strains of *S.aureus* produce several types of enterotoxins which can cause symptoms of intoxications such as vomiting, diarrhea and abdominal cramping (*Korpysaet al., 2005*).

Overall 200 samples examined, *S.aureus* was isolated from 13 , 14 and 12% of milk, kareish cheese and ice cream samples, respectively with mean counts of $5.8 \times 10^4 \pm 4.8 \times 10^3$, $11.9 \times 10^4 \pm 12.9 \times 10^3$ and $3.3 \times 10^4 \pm 2.6 \times 10^3$ (Tables 1 & 2). The highest frequency distribution (53.8%) lied within the range of ($10^4 - < 5 \times 10^4$);(85.8 %) within the range of ($5 \times 10^4 - < 5 \times 10^5$) and(83.3%) within the range of ($10^4 - < 5 \times 10^4$), respectively (Table 3). *S.aureus* is one of the most important pathogens that has received more attention due to its high incidence relatively to other organisms. Nearly similar results were obtained by *Kruy et al. (2001)*; *Hanaa(2008)* and *Mohammed (2009)*. Comparatively, lower values were declared by *Rajeev and Amit (2010)* and *Mohammed et al. (2012)*. Higher findings were recorded by *Saad (2013)* and *Saber (2013)*. *S.aureus* is one of the most prevalent bacteria in food poisoning in human(*Chiang et al., 2008*). In many countries, low degree contaminations by *S.aureus* are tolerated in most foodstuffs (up to 10^3 cfu/g in raw milk cheeses, in France), as they are not considered a risk for public health (*Le Loir et al., 2003*).

S.aureus enterotoxins (SEs) are produced during the exponential phase of *S.aureus* growth, With the quality being strain dependant . Typically, doses of SE that cause illness result when at least $10^5 - 10^8$ cfu/g of are present (*Seo and Bohach 2007; Montville and Matthews 2008*).

None of the examined samples exceeded the infectious dose required for *S.aureus* food poisoning. Many *S.aureus* outbreaks were recorded due to consumption of milk, cheese and ice-cream (*Hobbs and Roberts, 1987*).

In recent years much attention has been paid toward *E.coli* because of its importance as an organism of true faecal origin with the possible existence of associated enteric pathogens. Commensal *E.coli* plays a dynamic role in the ecology of intestinal tract. The *E.coli* genome exhibits a high degree of heterogeneity; therefore these bacteria can be a commensal organism. On the other hand it can be also a dangerous pathogen causing intestinal or extra-intestinal infections. Shiga toxin-producing *E.coli* (STEC) are one of the most important foodborne pathogens essential for public health, responsible for serious outbreaks, hemorrhagic colitis, and the hemolytic-uremic syndrome (HUS) and the leading cause of acute renal failure. Cattle is a natural reservoir of STEC and the transmission of this pathogen to humans can occur through the direct contact with infected animals, foods of animal origin such as meat or unpasteurized milk or via water contamination (*Etcheverría&Padola, 2013*). Also *E.coli* established to be among the etiological agents causing enteritis and several extra gastrointestinal diseases (*Robertson, 1988*). It is of major public health significance related to risk of introducing these bacteria to the food chain (*Newell et al., 2010*).

E. coli was detected in 11, 14 and 14% of milk, kareish cheese and ice cream samples, respectively (Table 1). The *E coli* counts ranged from

$1.2 \times 10^4 - 1.8 \times 10^5$; $1.3 \times 10^4 - 2.04 \times 10^5$ and $1.3 \times 10^4 - 8.1 \times 10^4$ with mean counts of $2.8 \times 10^4 \pm 1.5 \times 10^3$, $11 \times 10^4 \pm 11.3 \times 10^4$ and $3.6 \times 10^4 \pm 3.4 \times 10^3$, respectively (Table 2). The highest frequency distribution (72.8 %) lied within the range of ($10^4 - < 10^5$); (43%) within the range of ($10^5 - < 5 \times 10^5$) and (71.4%) within the range of ($10^4 - 5 < 10^4$) for milk, kareish cheese and ice cream samples, respectively (Table 4). *E. coli* is the most important coliform that has received more attention due to its high incidence relatively to other coliforms. Nearly similar results were obtained by *Hassan (2008)*; *Ahmed et al. (2009)* and *Maniruzzaman et al. (2010)*. Lower values were recorded by *Rajeev and Amit (2010)* and *AboZeed (2014)*. While, relatively higher values were declared by *Baraheemet al. (2007)*; *Lingathurai and Vellathurai (2010)* and *Ahmed, Esraa (2012)*. None of the examined samples exceeded the infectious dose (2.55×10^6) required for *E. coli* food poisoning (*Haas et al. 2000*)

Salmonella spp. was not detected in any of the examined samples. (Table1). These results were in accordance with that reported by *Mena (2011)* and *Abo Zeed(2014)*. But, it could be detected by *Hassan (2008)*; *Ahmed et al. (2009)* and *Mohammed et al. (2012)*. The presence of *Salmonella spp.* Indicated poor handling practices, sewage contamination, and the animal might be infected with mastitis and possibly from milk handlers (*I. CM. S. F., 1996* and *Yuen et al. (2012)*).

Salmonella have been considered to be the most important causal agents of foodborne illness throughout the world (*Majowicz et al., 2010; Hendriksen et al., 2011*). Raw milk is an important vehicle for *Salmonella* causing human infection. Hundreds of outbreaks in humans caused by milk products have primarily been due to the consumption of unpasteurized milk or cheeses made from unpasteurized milk (*Bell and Kyriakides, 2002*).

CONCLUSION

This study reports high prevalence of *S.aureus* and *E.coli* food poisoning organisms in Kafr EL-Sheikh governorate, Egypt. Therefore, special attention should be given to milk and dairy products, which act as invisible potential reservoir of these organisms that may constitute a public health hazard, and to guarantee safe consumption of raw milk and milk products.

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