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Prevalence and Significance of *Staphylococcus aureus* and Enterobacteriaceae species in Selected Dairy Products and Handlers

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Abstract: Some of the Egyptian dairy products are made by traditional methods. This makes them suitable media for multiplication of foodborne pathogens. So, this study aims to assess the microbial quality of selected dairy products prevalent in Ismailia City, the personal hygiene of dairy handlers and the potential hazards to consumers. A total of 120 Ras cheese, Kareish cheese and ice cream samples were collected randomly from different localities in Ismailia city. In addition, 40 nasal and hand swab samples were collected from some dairy workers. All samples were examined for presence of *Staphylococcus aureus* (on Baird Parker agar medium) and Enterobacteriaceae sp. (on Violet Red Bile Glucose agar medium). Molecular typing of *S. aureus* was performed using PCR Assay. The prevalence and significance of *Staphylococcus aureus* and Enterobacteriaceae species in Ras cheese, Kareish cheese and ice cream samples and in swabs of dairy handlers in Ismailia city were studied. The results of this study revealed out that the mean values of *S. aureus* counts (log_{10}cfu g^{-1}) in Ras cheese was 5.54, in Kareish cheese was 5.59 and in ice cream samples was 4.07. Meanwhile, the mean values of Enterobacteriaceae sp. counts (log_{10}cfu g^{-1}) in Ras cheese was 2.48, in Kareish cheese was 6.78 and in ice cream was 1.11. Salmonella could not be recovered in the examined dairy products. *Staphylococcus aureus* was isolated from 60 and 70% of dairy handlers hand's and nasal swab samples, respectively. Only one *Salmonella* strain was recovered from a dairy handler swab. Overall, the recovered *E. coli* serotypes from all the examined samples were O15:H11, O22:H5, O25: NM, O26:H11, O86:H34, O91:H10, O113:H21, O114:H2, O119:H6, O124:H7, O128:H2, O127:NM and O145:NM. In conclusion, the isolated serotypes constitute public health hazards to consumers.

Key words: *S. aureus*, Enterobacteriaceae, dairy products, handlers, public health hazard

INTRODUCTION

Ras cheese, Kareish cheese and ice-cream are considered the most popular Egyptian dairy products. Their manufacture and handling techniques in Egyptian markets are still primitive and unhygienic (Al-Ashmawy *et al*., 1994; Enas and Asmaa, 2001). Many contaminants find their way to raw milk, from which they gain access to dairy products (Joshi *et al*., 2004; Al-Khatib and Al-Mitwalli, 2009; Shathele, 2009; Sospedra *et al*., 2009; Zeinab *et al*., 2009).

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Foodborne diseases are a common and widespread global problem. Several outbreaks have been reported as a result of eating contaminated dairy food that may look, taste and smell perfectly normal but is in fact contaminated with large number of harmful bacteria (CDC, 2009).

Staphylococci are expected to be among the organisms that contaminate dairy products from handlers. Under favorable conditions, Staphylococci can grow and secrete heat resistant enterotoxins which cause vomiting and diarrhea within 30 min to 8 h after ingestion of incriminated foods.

Enterobacteriaceae sp. have been implicated in many cases of food poisoning outbreaks (Koneman *et al*., 1994). *Escherichia coli* is an important organism in the microbiology of the food, besides being involved in foodborne gastroenteritis, it is considered a good indicator of possible fecal contamination of dairy products (El-Bagoury and Mosaad, 2002).

Salmonella is considered among the most important enteric foodborne pathogen whose presence in the food constitutes a severe health hazard. Many outbreaks of human illness have been associated with the consumption of raw or inadequately heat treated milk or their dairy products (Ellis *et al*., 1998).

Therefore, the objectives of the present study were designed to determine the prevalence and public health significance of *S. aureus* and Enterobacteriaceae sp. in Ras cheese, Kareish cheese, ice-cream samples and in swabs from dairy handlers.

MATERIAL AND METHODS

Collection of the Samples

One hundred and twenty samples (40 samples each) of Ras cheese (250 g chilled in plastic bags), Kareish cheese (100 g in plastic bags from street vendors) and ice-cream (frozen in plastic cups, about 100 g) were obtained from different markets in Ismailia city. In addition, forty nasal and hand swab samples (20 samples each) were collected from dairy handlers. Each swab was put into a sterile tube containing 9 mL of sterile saline. Samples were transferred promptly to the laboratory in an ice-box for microbiological examination. The study was conducted during the period from September, 2008 to May, 2009 at the bacteriological laboratory of Food Hygiene Department, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Preparation of the Samples

The technique recommended by APHA (1992) was used for sample preparation. Twenty five grams of each cheese sample were aseptically added to 225 mL of sterile 2% sodium citrate solution and then blended in stomacher for 2 min to form a dilution of 1:10. Samples of ice-cream were thawed in a thermostatically controlled water bath at 44°C for not more than 15 min. Then, 25 g of each sample were aseptically mixed with 225 mL of 0.1% peptone water to form a dilution of 1:10. Tenth-fold serial dilutions were prepared using sterile 0.1% peptone water.

Microbiological Methods

Staphylococcus aureus was determined on Baird Parker agar medium (Oxoid), incubated at 37°C for 48 h according to APHA (1992). Enterobacteriaceae sp. were enumerated on Violet Red Bile Glucose agar medium (Oxoid), incubated at 37°C for 48 h according to APHA (1992). Salmonella and *E. coli* were recovered according to technique of USFDA (2001).

Table 1: Sequence and nucleotide positions of *Staphylococcus aureus* primers

Primer name	Sequence	Nucleotide position
16S rRNA FP	5'-GTA GGT GGC AAG CGT TAT CC-3´	545-564
16S rRNA RP	5´-CGC ACA TCA GCG TCA G-3´	773-758

Molecular Typing of *S. aureus* by PCR Assay

DNA template was prepared according to Sambrook *et al*. (1989). Identified colonies of *S. aureus* were subjected to DNA extraction by boiling method, a loopful of bacterial colony was suspended in 100 µL of PBS, Centrifugation occurred at 3000 rpm for 5 min, bacterial pellet was then dissolved in 100 µL of PBS and subjected to heating in a boiling water bath at 100°C for 10 min, then centrifugation at 13000 rpm for 15 min, the supernatant was transferred into a new sterile tube. The DNA purity and concentration were measured by spectrophotometer (Davis *et al*., 1986).

Oligonucleotide Primers (Monday and Bohach, 1999)

The sequences of the 16S rRNA from *S. aureus* were retrieved from GenBank (accession No. X68417). The oligonucleotide primers were designed by alignment of published DNA sequences of the *S. aureus* using Multian program (Corpet, 1988). Primers were purchased from the Biobasic Inc., Canada. The sequences and nucleotide positions of both forward and reverse primers were summarized in Table 1. These primers amplified a 228 bp amplicon from *S. aureus*.

Polymerase Chain Reaction

DNA samples (100 ng per reaction) were amplified in a 25 µL reaction mixture consisting of 1.5 unit Taq polymerase (Sibenzyme, Russia), 1 X TAQ polymerase buffer, 200 µM of dNTPs mixture, 20 pmole of each primers and sterile distilled water up to 25 µL. Amplification was performed in thermal cycler (Techne Progene, UK). Parameters for amplification included an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 64°C for 30 sec and extension at 72°C for 30 sec, followed by a final extension at 72°C for 5 min. Amplified products were separated by electrophoresis in a 1.7% agarose gel (Biobasic) stained by ethidium bromide (0.5 µg mL^{-1}), in 1 X TAE buffer at constant voltage of 4 v cm^{-1} and photographed with Sony digital camera. A 100 bp DNA marker (Axygen) was used as a DNA molecular size standard (Sharma *et al*., 2000).

Statistical Analysis

Chi square test was performed to analyze the epidemiologic data by using the Statistical Package for Social Sciences SPSS (version 10.0 for Windows; SPSS Inc., Chicago, IL. USA) and a p-value <0.05 was considered significant (Landau and Everitt, 2003).

RESULTS

Staphylococcus aureus and Enterobacteriaceae sp. in Examined Samples

It was clear in Table 2 that there was a highly significant difference ($\chi^2 = 14$, $p<0.001$) in the prevalence rates of *S. aureus* between the examined dairy product samples. The prevalence of *S. aureus* was 85% in Kareish cheese samples, followed by Ras cheese samples (80%) then ice-cream samples (50%). The mean values of *S. aureus* counts (log_{10} cfu g^{-1}) in Ras cheese was 5.54, in Kareish cheese was 5.59 and in ice cream samples was 4.07. The prevalence rates of *S. aureus* in hand and nasal swabs collected from dairy handlers were 60 and 70%, respectively (Table 5).

Table 2: Prevalence and counts (log_{10} cfu g^{-1}) of *S. aureus* and Enterobacteriaceae sp. in examined dairy products

	Microorganism									
	S. aureus					Enterobacteriaceae sp.				
Samples	No.	%	Min.	Max.	Mean	No.	%	Min.	Max.	Mean
Ras cheese	32	80	3	5.94	5.54	38	95	2.08	3.95	2.48
Kareish cheese	34	85	3	6.38	5.59	40	100	6.18	7.95	6.78
Ice-cream	20	50	3	4.70	4.07	30	75	0.95	3.34	1.11

No.: Number of positive samples ($\chi^2 = 14$, $p<0.001$)

Table 3: Prevalence of Enterobacteriaceae sp. in examined samples

	Samples							
	Ras cheese		Kareish cheese		Ice cream		Human hand swabs	
Microorganism	No.	%	No.	%	No.	%	No.	%
E. coli	7	29.2	4	44.4	7	31.8	12	44.4
E. adecarboxylata	1	4.2	4	44.4	1	4.5	-	-
E. blattae	-	-	-	-	3	13.6	-	-
Salmonella sp.	-	-	-	-	-	-	1	3.7
Enterobacter aerogenes	5	20.8	-	-	1	4.5	2	7.4
Klebsiella pneumoniae	5	20.8	1	11.1	4	18.2	5	18.5
Klebsiella oxytoca	-	-	-	-	4	18.2	-	-
Proteus vulgaris	1	4.2	-	-	-	-	2	7.4
Serratia species	5	20.8	-	-	2	9.1	-	-
Citrobacter freundii	-	-	-	-	-	-	5	18.5
Total	24	100.0	9	100.0	22	100.0	27	100.0

No.: Number of positive samples

The prevalence of Enterobacteriaceae sp. was 100% in Kareish cheese samples, followed by Ras cheese samples (95%) then ice-cream samples (75%). The mean values of Enterobacteriaceae counts (log_{10} cfu g^{-1}) in Ras cheese was 2.48, in Kareish cheese was 6.78 and in ice cream was 1.11.

Significance of the Isolated Enterobacteriaceae sp.

A total of 82 strains of the family Enterobacteriaceae were recovered from dairy products and handlers (Table 3, 4). The results showed that 7 (29.2%), 4 (44.4%), 7 (31.8%) and 12 (44.4%) strains of *E. coli* were recovered from Ras cheese, Kareish cheese, ice-cream and hand swab samples, respectively. Serotypes isolated from Ras cheese belonged to the STEC group (O22:H5, O91:H10 and O113: H21), the ETEC group (O15:H11) and 2 untypable strains. Serotypes isolated from Kareish cheese belonged to the STEC group (O128:H2), EPEC (O127:NM), EIEC group (O124:H7) and one untypable strain. Serotypes isolated from ice-cream belonged to the STEC group (O26:H11, O145:NM), EPEC group (O86:H34, O119:H6) and one untypable strain. Serotypes detected in hand swabs belonged to the STEC/EHEC group (O26:H11), EPEC group (O86:H36, O114:H2 and O119:H6), ETEC group (O25:NM) and EIEC group (O124:H7).

Other members of Enterobacteriaceae sp. were identified from the examined dairy products and hand swabs of dairy handlers (Table 3). Salmonella could not be isolated from the examined Ras cheese, Kareish cheese and ice-cream samples. Only one sample taken from a dairy handler confirmed the presence of *Salmonella* sp.

Molecular Typing of *S. aureus* Isolates

PCR protocol was used for amplification and detection of 16S rRNA genes of *S. aureus* isolates. Figure 1 illustrated the positive amplification of 228 bp fragment of 16S rRNA gene from the extracted DNA of *S. aureus* strains isolated from different sources.

Table 4: Prevalence of *E. coli* serotypes in relation to total number of *E. coli* isolates

	Samples							
	Ras cheese		Kareish cheese		Ice cream		Human hand swabs	
E. coli serotypes	No.	%	No.	%	No.	%	No.	%
1-STEC								
O22: H5	2	28.6	-	-	-	-	-	-
O26:H11	-	-	-	-	1	14.3	3	25.0
O91: H10	1	14.3	-	-	-	-	-	-
O113: H21	1	14.3	-	-	-	-	-	-
O128: H2	-	-	1	25	-	-	-	-
O145: NM	-	-	-	-	1	14.3	-	-
2-EPEC								
O86: H34	-	-	-	-	2	28.6	3	25.0
O114:H2	-	-	-	-	-	-	1	8.3
O119:H6	-	-	-	-	2	28.6	1	8.3
O127:NM	-	-	1	25	-	-	-	-
3-ETEC								
O15:H11	1	14.3	-	-	-	-	-	-
O25:NM	-	-	-	-	-	-	2	16.7
4-EIEC								
O124:H7	-	-	1	25	-	-	2	16.7
5-Untypable	2	28.6	1	25	1	14.3	-	-

No.: Number of positive samples, EHEC/STEC: Enterohemorrhagic *E. coli*/Shiga toxin producing *E. coli*, EPEC: Enteropathogenic *E. coli*, ETEC: Enterotoxigenic *E. coli*, EIEC: Enteroinvasive *E. coli*

Table 5: Prevalence of *S. aureus* in swabs of dairy handlers

Item	N	%
Hand swab (+) Nasal swab (+)	10	50
Hand swab (+) Nasal swab (-)	2	10
Hand swab (-) Nasal swab (+)	4	20
Hand swab (-) Nasal swab (-)	4	20
Total hand swab positive	12	60
Total nasal swab positive	14	70

+: Positive, -: Negative, N: Number of corresponding items

Fig. 1: Agarose gel electrophoresis pattern of 16S rRNA: 228 bp specific PCR product amplified with the forward and reverse primers, M: DNA molecular weight ladder (100 bp), Lane 1: Control positive *Staphylococcus aureus* strain, Lane 2: Control negative, Lane 3-9: Positive samples

DISCUSSION

It was clear that the mean value of *S. aureus* counts in the examined Ras cheese samples was $\log_{10}$ 5.54 cfu g^{-1}. Nazem and Thanaa (1993) recorded that the min., max. and mean

counts of *S. aureus* in Ras cheese were 1×10^2, 8.8×10^4 and 2.4×10^4 cfu g^{-1}, respectively. On the other hand, Al-Hawary *et al*. (2002) found that *Staphylococcus aureus* count ranged from 1×10^2 to 5.4×10^7 cfu g^{-1}.

In the current study, *Staphylococcus aureus* was detected in 85% of examined Kareish cheese samples. This result was higher than that reported by Kaldes (1997) and Azza *et al*. (2004) who reported that the prevalence rates of *Staphylococcus aureus* were 10 and 11.25% in fresh Kareish cheese samples, respectively. In addition, Bahout and Moustafa (2006) reported that *Staphylococcus aureus* was present in 28% of the examined Kareish cheese samples with min., max and mean counts of 1.1×10^2, 6.5×10^5 and 3.4×10^4 cfu g^{-1}, respectively.

In this study, *Staphylococcus aureus* was detected in 50% of examined ice cream samples. Azza *et al*. (2002) isolated *Staphylococcus aureus* from 100% of examined ice cream samples, the min., max. and mean counts were 9×10^3, 2.8×10^6 and 6.8×10^5 cfu g^{-1}, respectively. The high count of *Staphylococcus aureus* is indicative of poor hygienic measures during production, handling and distribution (Vought and Tatini, 1998; Joshi *et al*., 2004). The difference in the prevalence rates of *S. aureus* between the examined products may originate from the method of manufacture, storage and handling. Kareish cheese is made by farmers from raw milk that is not subjected to heat treatment. Street vendors put Kareish cheese in pans exposed to dust and flies. The lowest prevalence rate of *S. aureus* which was recorded in ice-cream might be attributed to the effect of freezing which inhibits the multiplication of this microorganism.

The growth of *S. aureus* in food represents a potential public health hazard since many strains of *S. aureus* produce heat resistant enterotoxins. Outbreaks of food poisoning occur when contaminated food is held at inappropriate temperature long enough to allow the bacteria to grow and release toxins (Adesiyun *et al*., 1998). Present study revealed out that the prevalence rates of *S. aureus* in hand and nasal swabs collected from dairy handlers were 60 and 70%, respectively. Food handlers are usually the main contamination source in food poisoning outbreaks through, skin, mouth, nose, respiratory infections and suppurative lesions. Therefore, *S. aureus* is a good indicator for the personal hygiene of the factory workers (Mahari and Gashe, 1990). Therefore, thoroughly hand washing before and after food preparation should be adopted. Food handlers who have skin infection should be prohibited from handling of food.

The current study pointed out that 95% of examined Ras cheese samples yielded Enterobacteriaceae sp. These results were confirmed by Nazem and Thanaa (1993) and Al-Hawary *et al*. (2002). It was clear that Enterobacteriaceae sp. were recovered in 100% of the examined Kareish cheese samples. Nearly similar results were recorded in 100 and 98.8% of examined samples by Ahlam (1998) and El-Kosi (2001), respectively. The Enterobacteriaceae sp. were detected in 75% of examined ice cream samples. Similar results were reported by Mansour *et al*. (2000). Higher results were confirmed by Amal *et al*. (2001) who reported that 100% of examined ice cream samples yielded Enterobacteriaceae. Enterobacteriaceae are distributed worldwide. They have medical and economic importance. Their presence in large numbers indicates faecal contamination of food, inadequate processing and post-processing contamination (Koneman *et al*., 1994).

No *Salmonella* could be detected in the examined dairy products. This agreed with (Al-Hawary *et al*., 2002; Bahout and Moustafa, 2006). On the contrary, Enas and Asmaa (2001), Azza *et al*. (2002) and El-Bagoury and Mosaad (2002) detected *Salmonella* sp. in dairy products. In this study, one sample taken from a dairy handler confirmed the presence of *Salmonella* sp., as a potent pathogen, it should be taken seriously.

Escherichia coli is the most common species of facultative anaerobe found in the human gastrointestinal tract and the most commonly encountered pathogen in the

Enterobacteriaceae family. Present result confirmed that Egyptian dairy products manufactured by traditional methods are regularly contaminated by *E. coli* and this agreed with (El-Essawy and Riad, 1990; Nazem and Thanaa, 1993; Kaldes, 1997; El-Kosi, 2001; El-Bagoury and Mosaad, 2002; Azza *et al*., 2004; Bahout and Moustafa, 2006; Sabry and Laila, 2008). *Escherichia coli* is the most common species of facultative anaerobe found in the human gastrointestinal tract and the most commonly encountered pathogen in the Enterobacteriaceae family. STEC/EHEC serotypes (particularly O26, O111, O128 and O103) comprise an important emerging group of zoonotic enteric pathogens of animals and humans and indeed may be more prominent than O157 (Bettelheim, 2007). In humans, some EHEC infections result in bloody or non bloody diarrhea, which may be complicated by haemorrhagic colitis and severe renal and neurological sequelae, including haemolytic uraemic syndrome (Lynn *et al*., 2005). EPEC are a leading cause of severe diarrhea in infants (Nguyen *et al*., 2006). ETEC are a major cause of traveler's and childhood diarrhea worldwide (Beatty *et al*., 2004). EIEC have been identified as a cause of diarrhea (Estrada-García *et al*., 2005).

The difference between our results and the previous studies may be attributed to sampling techniques, sources of samples (home or factory-made), handling of samples and types of media used.

It is considered that the *S. aureus* species specific PCR is useful for speeding up identification of *S. aureus* by replacing the current biochemical phenotypic schemes which are time consuming. Additionally, if appropriate conditions are established, direct PCR identification of *S. aureus* from food and clinical specimens can be performed (Jos *et al*., 1996).

In conclusion, our results revealed out that Egyptian Ras cheese, Kareish cheese and ice cream products sold in Ismailia city markets are contaminated with *S. aureus* and Enterobacteriaceae sp. These isolates constitute public health hazards to consumers. Periodical examination of dairy products to ensure safety for consumers must be practiced. The isolation of bacterial pathogens from dairy workers reflects bad hygienic standards and necessitates regular inspection of them for prevalence of foodborne pathogens. Adoption of reward and punishment policy may help to improve their hygienic standards. Overall, good quality raw materials used in product processing, adoption of Good Manufactured Practices (GMP) and strict personal hygiene are the way to ensure safety and high quality dairy products.

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