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Research Article

Identification of *S.aureus* and *E.coli* from Dairy Products Intended for Human Consumption

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Abstract | The present study was performed to determine the prevalence of *S. aureus* and *E. coli* in milk and some dairy products sold in Mansoura city, Egypt. A total two hundred samples including kariesh cheese (50 samples), market raw milk, market sterile milk, small scale yoghurt, large scale yoghurt, small scale ice cream, and large scale ice cream samples (25 samples from each product) were collected for microbiological examination. Recovered isolates were identified using an array of biochemical and serological tests for *E. coli*. Polymerase chain reaction (PCR) was applied to detect the *nuc* gene as a species-specific marker gene for *S.aureus*. Our result revealed that *E. coli* was observed in 86%, 60%, 88%,80%, 92% and 28% of kariesh cheese, market raw milk, small scale yoghurt, large scale yoghurt, small scale ice cream and large scale ice cream samples, respectively.None of the sterile milk samples was recorded for *E. coli* contamination. The prevalence of *S. aureus* was 92%, 80%, 36%, 100% and 40%, respectively. Likewise, none of the yoghurt large scale & sterile milk samples was recorded for *S. aureus* contamination. These findings highlighted the importance of periodical application of strict hygienic measures in dairy plants and markets for monitoring the public health hazards.

Keywords | *E.coli*, *S.aureus*, Milk, Dairy products.

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INTRODUCTION

The nutritional richness of milk is a goods ource of high biological value proteins and important vitamins and essential minerals (Pereira, 2014). Kareish cheese is one of the most popular cheese varieties consumed in Egypt especially in countryside due to its high protein, low fat and reasonable price (Metwalli, 2011). Likewise, yoghurt is considered as an excellent source of protein,vitamins, minerals and all the amino acids essential to heath (Mckinley, 2005). Ice cream is frequently considered as a 'fun food,' which is undeserving consideration. In reality, ice cream is a relatively well-balanced, healthy, easily digestible, and delicious food (Deosarkar et al., 2016). Milk & dairy products serves as very good medium for the growth of many microorganisms including pathogenic bacteria (Ruegg, 2003) accounting for 5% of all the incriminated foods in staphylococcal outbreaks, referring to Europe (Bianchiet al., 2014). Therefore milk & dairy products must be manufactured, stored and distributed according to good hygienic practices to avoid deterioration of the products and health hazards of consumers. Therefore, this study was carried out to highlight the microbiological status of milk and dairy products samples sold in Mansoura City, Egypt, specifically the prevalence of the most important food-borne pathogens *S. aureus* and *E. coli*.

MATERIALS AND METHODS

Collection and Preparation of Samples

Two hundred sampleswere collected for microbiological examination, including 50 milk samples (including 25

market raw milk samples and 25 market sterile milk samples), 50 kariesh cheese, 50 ice cream (including 25 small scale samples and 25 large scale samples), and 50 yoghurt (including 25 small scale samples and 25 large scale samples) (Table 1).

Table 1: Collection of the different samples for examination

Area of collection	No. of samples	Products
Supermarkets in Mansoura city, Egypt	50	Kariesh cheese
Retail dairy shops in Mansoura city, Egypt	25	Raw market milk
Supermarkets in Mansoura city, Egypt	25	Sterile market milk
Retail dairy shops in Mansoura city, Egypt	25	Yoghurt small scale
Supermarkets in Mansoura city, Egypt	25	Yoghurt large scale
Ice cream shops in Mansoura city, Egypt	25	Ice cream small scale
Supermarkets in Mansoura city, Egypt	25	Ice cream large scale

Microbial Examination

Identification and characterization of *E. coli*: Suspected isolates were confirmed by biochemical tests as published (Kreig and Holt, 1984). Serological identification was carried out by using rapid diagnostic *E.coli* antisera sets (Denka Seiken Co, Japan) for diagnosis of the Enteropathogenic types (Koki et al., 1996).

Identification and characterization of *S. aureus*: Colonies with typical morphology of *S. aureus* were streaked on Baired- parker agar medium for purification (APHA, "American Public Health Association" 1992). Isolates were examined morphological identification by Gram`s stain (Cruickshank et al., 1975), biochemical tests (Arora, 2003) and identified by PCR for detection of *nuc* gene for detection of *S. aureus* gene (Sallam et al., 2015) using the primer sequence outlined in Table 2.

Table 2: Primer sets for PCR amplification of *nuc* gene specific for molecular identification of *S.aureus*.

Target gene	Primer direction and sequence	Amplicon size (bp)	Source
Nuc	F:5´-GTGCTGGCATATGTATGGCAATTG-3´ R:5´-CTGAATCAGCGTTGTCTTCGCTCCAA-3´	660	Sallam et al., 2015.

Statistical Analysis

Statistical analysis was performed by the (SAS, 2004) computer program using the general linear models (GLM). When the F-statistic was significant ($P < 0.05$), a mean separation was performed using the least significant difference (LSD) test.

RESULTS

In this study *E. coli* was detected on EMB agar among all milk and dairy products with mean counts $1.4\times10^5\pm1.9\times10^4$, $2.0\times10^5 \pm 4.4\times10^4$, $1.2\times10^4\pm 2.0\times10^3$, $1.0\times10 \pm 2.1$, $2.3\times10^3 \pm 4.9\times10^2$ and $4.4\times10^3 \pm 9.7\times10^2$ colony-forming units (CFU)/g or mlin kareish cheese, market raw milk, yoghurt small scale, yoghurt large scale, ice cream small scale and ice cream large scale samples, respectively. *E. coli* was not found in market sterile milk (Figure 1). The recognized *E. coli* strains from different dairy products biochemically identified *E. coli* isolates were sent to Food Analysis Center, at Faculty of veterinary medicine, Benha University in Egypt for serological identification and results of serological examination is outlined in Table 3. *S.aureus* was detected respectively with mean counts $2.4\times10^5\pm4.6\times10^4$, $1.2\times10^5\pm 2.2\times10^4$, $5.9\times10^3\pm 1.1\times10^3$, $1.9\times10^3\pm 1.9\times10^2$ and $1.1\times10^3 \pm 4.7\times10^2$ colony-forming units (CFU)/g or ml Figure 2 among tested kareish cheese, market raw milk, ice cream large scale, ice cream small scale, and yoghurt small scale samples respectively. Large scale yoghurt and market sterile milk was found negative for *S. aureus*. These results were confirmed by PCR detection of *nuc* gene as a species-specific marker gene for *S.aureus* (Figure 3). According to the results outlined it was obvious that large amount of dairy products available to the consumers that was not matching with the Egyptian standard (EOSQC, 2005) presented in Table 4 and Table 5.

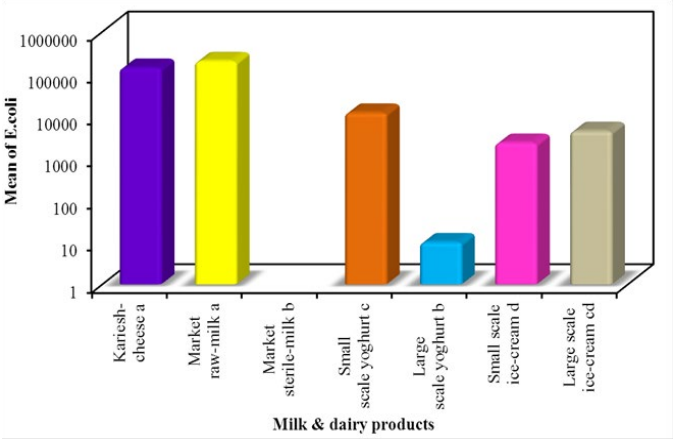

Figure 1: Statistical analytical results of *E. coli* in milk and some dairy products on EMB.

DISCUSSION

In the present study, the highest frequency of *E. coli* in kariesh cheese was 86% which lies within the range of $10^4 \leq$

Table 3: Serological identification of representative isolates of *E. coli* (n=30) from milk & dairy products.

Strain Characterization	Serodiagnosis	Positive Samples		Types of examined samples
		%	No. of isolated (30)	
ETEC	O127: H6	6.66	2	Representative samples of Milk & Dairy products n=30
	O128: H2	13.33	4	
	O111: H2	13.33	4	
EHEC	O26: H11	16.66	5	
	O121: H7	3.33	1	
	O103: H2	3.33	1	
EIEC	O124	6.66	2	
EPEC	O153: H21	3.33	1	
	O44: H18	6.66	2	
	O6: H4	6.66	2	
	O119: H6	6.66	2	
	O15	3.33	1	
		90	27	Total

Table 4: Quality of dairy products according toEOSQC 1008-2005 for presence of *E.coli* on EMB agar.

Unacceptable		Acceptable		Standard	No. of samples	Products
%	No.	%	No.			
86	43	14	7	Free	50	Kariesh cheese
60	15	40	10	Free	25	Raw market milk
0	0	100	25	Free	25	Sterile market milk
88	22	12	3	Free	25	Yoghurt small scale
80	20	20	5	Free	25	Yoghurt large scale
92	23	8	2	Free	25	Ice cream small scale
28	7	72	18	Free	25	Ice cream Large scale

Table 5: Quality of dairy products according to EOSQC 1008-2005 for presence of *S. aureus* on Baired parker agar.

Unacceptable		Acceptable		Standard	No. of samples	Product
%	No.	%	No.			
92	46	8	4	Free	50	Kariesh cheese
80	20	20	5	<100	25	Raw market milk
0	0	100	25	Free	25	Sterile market milk
36	9	64	16	Free	25	Yoghurt small scale
0	0	100	25	Free	25	Yoghurt large scale
100	25	0	0	Free	25	Ice cream small scale
40	10	60	15	Free	25	Ice cream large scale

10^6. In Egypt, different reports highlighted the contamination of Kareish cheese with *E. coli* acting as a public health hazard (Ombarak et al., 2016) as this type of cheese is homemade and sold from door to door or sold in local markets (FAO, 1999). Different sources might be implicated in microbial contamination of kariesh cheese as starting with using poor quality of raw milk, processing under uncontrolled environments and selection of the inappropriate starter culture for the fermentation (Awad, 2016). Raw Milk serves as an excellent medium for the growth of many microorganisms and the highest frequency distribution of *E. coli* in raw milk was 60% lies within the range of $10^4 \leq 10^6$. Obviously, occurrence of *E. coli* in milk may arise from machines, manual milking and inferior quality of water (Chye et al., 2004). In this study, *E.coli* was not detected in sterile milk, suggesting their excellent sanitary quality as previously observed elsewhere (Gamal et al.,2015). The frequency distribution of *E. coli* in yoghurt was 88% in small scale which lies within the range of $10^3 \leq 10^5$and s 80% for large scale which lies within $<10^3$. Presence of *E.c*

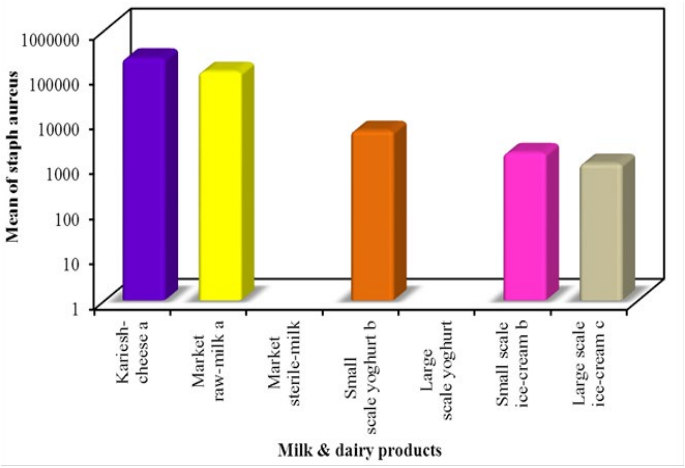

Figure 2: Statistical analytical results of *S. aureus* in milk and some dairy products on Baird parker medium.

Figure 3: Representative Agarose gel electrophoresis of PCR amplicons of the marker genes identified in *S. aureus* isolates from milk Dairy products samples. Amplified bands of the expected sizes of 660 bp for *nuc* gene**.**

-oli suggested post-pasteurization contamination of the product prior to or during packaging (Omola et al., 2014). Furthermore, these results suggested a general lack of cleanliness in handling and improper storage (Shojaei and Yadollahi, 2008). In ice cream, the frequency distribution of *E. coli* was 92% for small scale which lies within $10^3 \leq 10^5$ and in 28% in large scale which lies within $10^3 \leq 10^4$. Contamination can occur from different source include poor sanitary practices during processing and the use of contaminated components, mainly shell eggs and contaminated water (Walker et al., 1990).This difference in isolation rates might be attributed to different procedures used in packing and marketing of ice cream (Ambily and Beena, 2012). *E. coli* are harmless but some are known to be pathogenic bacteria, causing sever intestinal diseases in man (Kaper et al., 2004) and some strains are multiple drug resistant (MDR), this alarms the need for devising appropriate strategies to prevent the spread of resistance (Annal Selva- Malar et al., 2018). The highest frequency distribution of *S. aureus* in kariesh cheese was 92% whichlies within the range of $10^4 \leq 10^7$. Contamination can occur from different source include the poor quality of raw milk and processing in uncontrolled environments (Sameh, 2016). The highest frequency distribution of *S. aureus* in raw milk was 80% whichlies within the range of $10^4 \leq 10^6$. In sterile milk samples, *S. aureus*wasnot detected in all examined samples. Indicating that their excellent sanitary quality.The highest frequency distribution of *S. aureus* in small scale yoghurt was 36% which lies within the range of $10^3 \leq 10^5$. Presence of *S. aureus* in yoghurt usually indicates contamination from food handlers (Hussain, 2010). In the current study, the absence of bacterial contamination among large scale yoghurt suggested good sanitary quality. The highest frequency distribution of *S. aureus* in small scale ice cream was 40% which lies within the range of $10^3 \leq 10^4$, but in large scale ice cream cannot be detected. The isolation rates of *S. aureus* vary from investigator to another due to variable components used in preparation of ice cream (Reij and Den-Aantrekker, 2004). It was obvious that large amount of dairy products available to the consumers did not agree with Egyptian standard. This is referred to what degree of these products exposed to contamination during various stages of production.

CONCLUSION

Overall, these findings suggested that microbiological quality of raw milk and dairy products were highly contaminated with *E.coli.* and *S.aureus*. This indicates improper hygienic measures. Therefore, establishment of GMP "good manufacturing practice" and HACCP system in dairy plants and strict hygienic measures should be applied.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

AA and MA conceived and designed the study, performed the tests, analyzed and interpreted the data, and wrote the manuscript. ME and AE substantially contributed in analysis of the results and contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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