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## Isolation of enterotoxigenic *Staphylococcus aureus* harboring *seb* gene and enteropathogenic *Escherichia coli* (serogroups O18, O114, and O125) from soft and hard artisanal cheeses in Egypt

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### Abstract

**Background:** Soft and hard artisanal cheeses are regularly consumed in Egypt. These products are usually processed from raw milk which may harbor many pathogenic and spoilage microorganisms.

**Aim:** To evaluate the safety of some artisanal cheeses in Egypt, such as Ras, Domiati, and Mish, through chemical and microbiological examination.

**Methods:** One hundred and fifty random samples of traditional Ras, Domiati, and Mish cheeses (50 each) were microbiologically and chemically analyzed. Counts of total bacteria, presumptive coliform, staphylococci, yeast, and mold were estimated. Furthermore, isolation of *Escherichia coli* and *Staphylococcus aureus* was performed, followed by PCR confirmation; isolates of *E. coli* were examined for the presence of virulence genes; on the other hand, the detection of the five classical enterotoxin genes of *S. aureus* was performed using multiplex PCR. Regarding chemical analysis, moisture, salt, and acidity content were measured. Correlations between chemical and microbial findings were investigated.

**Results:** Mean counts of total bacteria, presumptive coliform, staphylococci, yeast, and mold were ( $2 \times 10^8$ ,  $3 \times 10^6$  and  $1 \times 10^7$ ), ( $3 \times 10^5$ ,  $5 \times 10$  and  $5 \times 10^2$ ), ( $1 \times 10^6$ ,  $4 \times 10^5$  and  $1 \times 10^5$ ), ( $3 \times 10^5$ ,  $1 \times 10^5$  and  $5 \times 10^5$ ), and ( $7 \times 10^3$ ,  $4 \times 10^3$  and  $3 \times 10^4$ ) for Ras, Domiati and Mish cheeses, respectively. Serological identification of suspected *E. coli* revealed that *E. coli* O125 was isolated from Ras and Domiati samples, *E. coli* O18 was recovered from Ras samples, while *E. coli* O114 was isolated from Mish samples. PCR results revealed that all detected isolates of *E. coli* were positive for both *iss* (increased serum survival) and *fimH* (type 1 fimbriae) genes. Concerning isolated *S. aureus*, all examined products were harboring *S. aureus* enterotoxigenic strains, with *seb* and *sed* genes being the most common. The mean values of moisture, salt, and acidity were (30.03, 56.44, and 58.70), (3.30, 6.63, and 7.56) and (0.65, 0.68, and 0.50) for Ras, Domiati, and Mish cheeses, respectively.

**Conclusion:** Enterotoxigenic *S. aureus* harboring *seb* gene and enteropathogenic *E. coli* (serogroups O18, O114, and O125) were frequently isolated from soft and hard artisanal cheeses in Egypt. Therefore, strict hygienic measures should be applied during their manufacture, handling, and distribution.

**Keywords:** Domiati, *E. coli* virulence genes, Mish, Ras, *S. aureus* enterotoxins.

### Introduction

Traditional Egyptian dairy products are an essential part of the daily diet in Egypt. Raw milk is the common factor in making all traditional products, which usually record high sensorial characteristics, as consumers mostly choose their dairy products based on its sensory properties, regardless if it is made from raw or pasteurized milk (Awad, 2006; El-Ghaish *et al.*, 2010; Ewida and Hussein, 2019). Some traditional products have been traced back to the pharaoh era of 4,000 BC, and is then handed down from one generation to another. There are different types of traditional cheese in Egypt and among them are Ras, Domiati, and Mish (Benkerroum, 2012).

Ras cheese is a hard cheese commonly known in Egypt as “Romi”. It is one of the artisan type cheeses, which is produced often in small factories located in rural

areas. It is mainly made from raw milk, either cow’s or a mixture of cow and buffalo milk, mostly without adding a starter culture, and fermentation and ripening occur only due to the native flora of raw milk, then it is kept to ripen for 3–8 months till a sharp flavor is acquired, similar to the Greek Kefalotyri cheese (Awad, 2006; El-Fadaly *et al.*, 2015).

Domiati or Damietta cheese (Gebnah Domiata) is one of the most popular white, soft pickled cheeses. It is named after the Egyptian seaport city “Dumyât”. Typically, it is salty, unlike other pickled cheeses, as the salt is added directly to the raw milk at the proportion of 5%–14%, which varies according to season and ripening conditions (Robinson and Tamime, 1991).

Mish is one of the oldest indigenous milk products in Egypt that has a sharp harsh flavor with a relatively higher salt content and is mostly yellowish brown color.

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Karish cheese is considered as the basic raw material for such a product. Some other ingredients that may be included in Mish are sour milk, buttermilk, morta (a byproduct of ghee), different types of pepper (green, red, and paprika), table salt, and a portion of an old Mish which acts as the starter culture. All ingredients are brined under microaerophilic conditions mostly in earthenware containers for more than 1 year to obtain matured mish, which consists of Mish cheese and Mish slurry (pickling medium), and both are consumed (Tamime, 2006).

Raw milk used in the production of traditional dairy products and harbor a large number of native microflora that may participate in the development of a desirable aroma and an appropriate flavor, but it may also contain pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, and others that are considered as public health hazards, causing a serious food illness and may produce lethal toxins in the food (Awad et al., 2003; El-Ghaish et al., 2010). Moreover, undesirable spoilage organisms may also be found that cause defects and limits the shelf life of these products, rendering them unmarketable. Many other factors may cause an increase in the microbial load of traditional products, such as manufacture, handling, storage, and even marketing ways, which are still primitive and unhygienic (Osman et al., 2011).

The current study was conducted to assess the safety of some traditional cheeses in Egypt, such as Ras, Domiati, and Mish, through chemical and microbiological examination.

## Materials and Methods

### Samples collection

One hundred and fifty random samples of Ras, Domiati, and Mish cheeses (50 each) were collected from different Egyptian Governorates. The samples were identified, placed in an ice box, and examined immediately.

### Chemical analysis

Moisture and acidity were analyzed according to the AOAC guidelines (Association of Official Analytical Chemists) (2000) and salt according to the APHA guidelines (American Public Health Association) (2004).

### Microbiological examination

Preparation of food homogenate and decimal dilutions, total bacterial count (TBC; CFU/g), presumptive coliform count (MPN/g), and isolation of *E. coli* were carried out according to the APHA guidelines (American Public Health Association) (2004).

Biochemical identification of *E. coli* (microscopic examination, oxidase, urea, IMVC, TSI, L-Lysin decarboxylase, carbohydrate fermentation tests, and confirmed tests for thermotolerant (fecal) coliforms) was carried out according to Silva et al.'s (2019) description.

Serological identification of *E. coli* was done using DENKA SEIKEN CO., LTD. kits. The molecular characterization, and virulence genes detection was performed using PCR, where DNA was extracted from the bacterial isolates using QIAamp® DNA Mini Kit (Catalogue no.51304) according to the instruction manual. PCR was done for characterization of 5 genes of *E. coli* using Emerald Amp GT PCR master mix, Takara (Catalogue No. RR310A), using 5 different pair of primers (as uniplex PCR) as mentioned in Table 1. The PCR products were electrophoresed in 1.5% agarose according to that described in Sambrook et al.'s (1989) study.

Staphylococci count (CFU/g) and *S. aureus* isolation were carried out according to the APHA guidelines (American Public Health Association) (2004).

Identification of suspected *S. aureus* strains (microscopic examination, catalase activity test, coagulase test, thermostable nuclease, and anaerobic utilization of mannitol) was carried out according to that described in Silva et al.'s (2019) study. Molecular characterization of *S. aureus* strains and enterotoxin genes detection was done as in *E. coli*. Six genes of *S. aureus* (5 enterotoxins done by multiplex PCR and 23S rRNA) and six pairs (5 as multiplex and 1 pair as uniplex PCR) were used (Table 1).

Yeast and mold counts (CFU/g) were conducted according to APHA's guidelines (American Public Health Association) (2004).

### Statistical analysis

The collected data were analyzed using Statistical Package for the Social Sciences Statistics 17 for Windows.

### Ethical approval

In this study, no experimental live animals were used. All cheese samples were collected directly from the Egyptian local markets.

## Results

### Chemical analysis

The chemical analysis of the examined samples, including moisture, salt, and acidity, is illustrated in Table 2; for Ras cheese samples, the mean values were 30.03, 3.30, and 0.65, respectively. The mean values of Domiati cheese samples were 56.44, 6.63, and 0.68, respectively, and were 58.70, 7.56, and 0.50, respectively, for Mish samples.

### Microbiological examination

As reported in Table 3, the TBCs of Ras, Domiati, and Mish samples were  $2 \times 10^8$ ,  $3 \times 10^6$ , and  $1 \times 10^7$ , respectively, while coliforms were determined in 44%, 48%, and 92% of that examined in Ras, Domiati, and Mish samples, with a mean values of  $3 \times 10^5$ ,  $5 \times 10^4$ , and  $5 \times 10^2$ , respectively. The examined samples were positive for *E. coli* in 8% of Ras cheese samples and in 4% of both Domiati and Mish samples, respectively, as shown in Table 5. The serological

**Table 1.** Oligonucleotide primers' sequences and PCR conditions.

Target agent	Length of amplified product	Primer sequence (5'-3')	Gene	Reference	
<i>S. aureus</i>	102 bp	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	<i>sea</i>	Mehrotra <i>et al.</i> , 2000x	
	278 bp	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTCGTTT	<i>sed</i>		
	209 bp	AGGTTTTTTCACAGGTCATCC CTTTTTTCTTCGGTCAATC	<i>see</i>		
	164 bp	GTATGGTGGTGTAAGTACGAGC CCAAATAGTGACGAGTTAGG	<i>seb</i>		
	451 bp	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	<i>sec</i>		
	Primary denaturation: 94°C/5 minutes; secondary denaturation: 94°C/30 seconds; annealing: 57°C/40 seconds; extension: 72°C/45 seconds; No. of cycles: 35; final extension: 72°C/10 minutes.				
1250 bp	AC GGAGTTACAAAGGACGAC AGCTCAGCCTTAACGAGTAC	23S rRNA	Straub <i>et al.</i> , 1999		
	Primary denaturation: 94°C/5 minutes; secondary denaturation: 94°C/30 seconds; annealing: 55°C/40 seconds; extension: 72°C/1.2 minutes.; No. of cycles: 35; final extension: 72°C/12 minutes.				
<i>E. coli</i>	266 bp	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATAACC	Iss	Yaguchi <i>et al.</i> , 2007	
		Primary denaturation: 94°C/5 minutes; secondary denaturation: 94°C/30 seconds; annealing: 54°C/30 seconds; extension: 72°C/30 seconds; No. of cycles: 35; final extension: 72°C/7 minutes.			
	614 bp	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	Stx1	Dipineto <i>et al.</i> , 2006	
		Primary denaturation: 94°C/5 minutes; secondary denaturation: 94°C/30 seconds; annealing: 58°C/40 seconds; extension: 72°C/45 seconds; No. of cycles: 35; final extension: 72°C/10 minutes.			
	508 bp	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	fimH	Ghanbarpour and Salehi, 2010	
		Primary denaturation: 94°C/5 minutes; secondary denaturation: 94°C/30 seconds; annealing: 50°C/40 seconds; extension: 72°C/45 seconds; No. of cycles: 35; final extension: 72°C/10 minutes.			
	620 bp	GGT GGT GCA CTG GAG TGG AGT CCA GCG TGA TAG TGG	Tsh	Delicato <i>et al.</i> , 2003	
	Primary denaturation: 94°C/5 minutes; secondary denaturation: 94°C/30 seconds; annealing: 54°C/40 seconds; extension: 72°C/45 seconds; No. of cycles: 35; final extension: 72°C/10 minutes.				
720 bp	CGATTCTGGAAATGGCAAAG CGTGATCAGCGGTGACTATGAC	phoA	Hu <i>et al.</i> , 2011		
	Primary denaturation: 94°C/5 minutes; secondary denaturation: 94°C/30 seconds; annealing: 55°C/40 seconds; extension: 72°C/45 seconds; No. of cycles: 35; final extension: 72°C/10 minutes.				

**Table 2.** Chemical analysis of examined samples (*n* = 50 each).

Samples	Moisture %			Salt %			Acidity %		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
Ras cheese	22.35	39.96	30.03 ± 0.59	2.00	4.40	3.30 ± 0.10	0.36	0.97	0.65 ± 0.02
Domiat cheese	48.92	62.92	56.44 ± 0.50	3.51	8.78	6.63 ± 0.20	0.36	2.16	0.68 ± 0.04
Mish	43.67	69.38	58.70 ± 0.97	3.51	9.33	7.56 ± 0.29	0.14	1.44	0.50 ± 0.05

**Table 3.** Microbiological evaluation of examined samples (CFU/g) (*n* = 50 each).

		Ras cheese	Domiaty cheese	Mish
Total bacterial count	<b>No. (%) of positive samples</b>	<b>50 (100%)</b>	<b>50 (100%)</b>	<b>50 (100%)</b>
	Min.	$5 \times 10^5$	$7 \times 10^4$	$7 \times 10^3$
	Max.	$1 \times 10^9$	$1 \times 10^7$	$1 \times 10^8$
	Mean $\pm$ SE	$2 \times 10^8 \pm 0.5 \times 10^8$	$3 \times 10^6 \pm 0.4 \times 10^6$	$1 \times 10^7 \pm 0.2 \times 10^7$
Coliforms count (MPN/g)	<b>No. (%) of positive samples</b>	<b>22 (44%)</b>	<b>24 (48%)</b>	<b>46 (92%)</b>
	Min.	$2 \times 10^4$	$4 \times 10$	$2 \times 10$
	Max.	$2 \times 10^6$	$2 \times 10^2$	$2 \times 10^3$
	Mean $\pm$ SE	$3 \times 10^5 \pm 1 \times 10^5$	$5 \times 10 \pm 0.1 \times 10$	$5 \times 10^2 \pm 0.8 \times 10^2$
<i>Staphylococcus</i> count	<b>No. (%) of positive samples</b>	<b>46 (92%)</b>	<b>44 (88%)</b>	<b>50 (100%)</b>
	Min.	$1 \times 10^4$	$1 \times 10^4$	$7 \times 10^2$
	Max.	$6 \times 10^6$	$2 \times 10^6$	$9 \times 10^5$
	Mean $\pm$ SE	$1 \times 10^6 \pm 0.2 \times 10^6$	$4 \times 10^5 \pm 0.7 \times 10^5$	$1 \times 10^5 \pm 0.2 \times 10^5$
Yeast count	<b>No. (%) of positive samples</b>	<b>45 (90%)</b>	<b>50 (100%)</b>	<b>46 (92%)</b>
	Min.	$1 \times 10^4$	$3 \times 10^3$	$4 \times 10^3$
	Max.	$1 \times 10^6$	$8 \times 10^5$	$1 \times 10^6$
	Mean $\pm$ SE	$3 \times 10^5 \pm 0.6 \times 10^5$	$1 \times 10^5 \pm 0.2 \times 10^5$	$5 \times 10^5 \pm 0.6 \times 10^5$
Mold count	<b>No. (%) of positive samples</b>	<b>25 (50%)</b>	<b>22 (44%)</b>	<b>36 (72%)</b>
	Min.	$2 \times 10^3$	$1 \times 10^3$	$3 \times 10^2$
	Max.	$2 \times 10^4$	$2 \times 10^4$	$1 \times 10^5$
	Mean $\pm$ SE	$7 \times 10^3 \pm 0.9 \times 10^3$	$4 \times 10^3 \pm 0.9 \times 10^3$	$3 \times 10^4 \pm 0.7 \times 10^4$

**Table 4.** Statistical correlation between chemical and microbiological analyses.

	Moisture %		Salt %		Acidity %	
	Pearson correlation	Sig.	Pearson correlation	Sig.	Pearson correlation	Sig.
Ras cheese						
TBC	0.215	0.134	-0.151	0.295	-0.171	0.235
Coliforms count	0.430 <sup>a</sup>	0.002 <sup>a</sup>	-0.394 <sup>a</sup>	0.005 <sup>a</sup>	-0.048	0.739
Staph. count	0.182	0.207	-0.197	0.170	-0.088	0.542
Yeast count	0.067	0.644	-0.070	0.630	-0.169	0.240
Mold count	0.169	0.241	-0.094	0.518	-0.078	0.589
Domiaty cheese						
TBC	0.326 <sup>b</sup>	0.021 <sup>b</sup>	-0.170	0.237	-0.237	0.097
Coliforms count	0.229	0.110	-0.806 <sup>a</sup>	0.000 <sup>a</sup>	-0.103	0.475
Staph. count	0.388 <sup>a</sup>	0.005 <sup>a</sup>	-0.267	0.061	-0.391 <sup>a</sup>	0.005 <sup>a</sup>
Yeast count	0.115	0.427	-0.306 <sup>b</sup>	0.031 <sup>b</sup>	-0.245	0.086
Mold count	0.062	0.668	-0.142	0.327	-0.256	0.073
Mish						
TBC	0.423 <sup>b</sup>	0.022 <sup>b</sup>	-0.169	0.381	-0.047	0.807
Coliforms count	0.039	0.788	-0.590 <sup>a</sup>	0.000 <sup>a</sup>	-0.165	0.215
Staph. count	0.131	0.364	-0.315 <sup>b</sup>	0.026 <sup>b</sup>	-0.516 <sup>a</sup>	0.000 <sup>a</sup>
Yeast count	0.556 <sup>a</sup>	0.000	-0.221	0.123	-0.481 <sup>a</sup>	0.000 <sup>a</sup>
Mold count	0.054	0.707	-0.144	0.317	-0.259	0.070

<sup>a</sup>Correlation is significant at the 0.01 level (2-tailed).

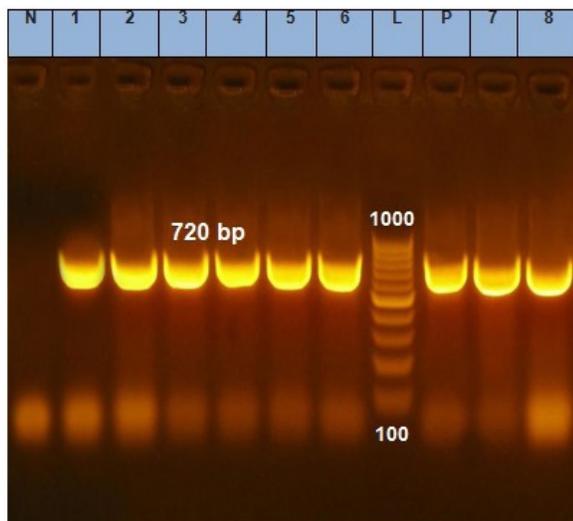
<sup>b</sup>Correlation is significant at the 0.05 level (2-tailed).

identification of the suspected isolated *E. coli* using slide agglutination test (Table 5) revealed that *E. coli* O125 was isolated from Ras and Domiati cheese samples; *E. coli* O18 was isolated from Ras samples, while *E. coli* O114 was detected in Mish samples. The molecular characterization of *E. coli* showed that all *E. coli* strains isolated from the present study were positive for *phoA* gene (Fig. 1). All isolates of *E. coli* lacked both *stx1* and *tsh* genes; on the other hand, all detected isolates were positive for both *iss* and *fimH* genes (Figs. 2–5). Staphylococci were found in 92%, 88%, and 100% of Ras, Domiati, and Mish samples with mean values of  $1 \times 10^6$ ,  $4 \times 10^5$ , and  $1 \times 10^5$ , respectively (Table 3). The incidences of *S. aureus* in the examined Ras, Domiati, and Mish samples based on biochemical identification were 26%, 36%,

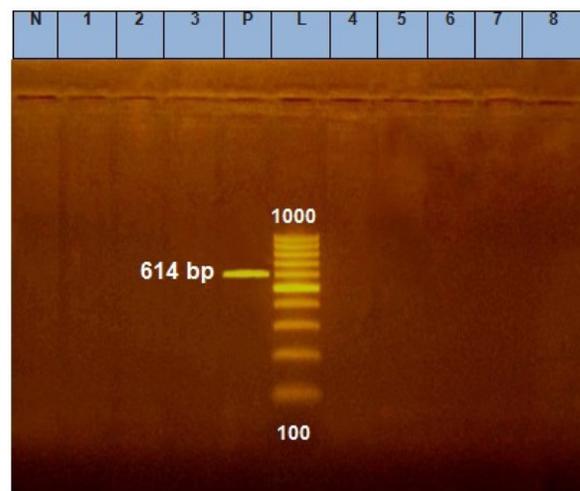
and 18%, as presented in Table 6. The number of molecularly identified *S. aureus* isolates by using 23S rRNA for the examined Ras, Domiati, and Mish samples was 2, 13, and 6, respectively (Fig. 6). The detection of the five classical enterotoxin genes of *S. aureus* revealed that the two strains identified from Ras cheese were enterotoxigenic strains having *seb* and *sed* genes; in Domiati samples, 10 strains were enterotoxigenic strains harboring *seb*, *sed*, and *see* genes, while the 6 strains from Mish samples were enterotoxigenic strains harboring *seb* gene (Fig. 7). The mean values of yeast / mold counts of the examined Ras, Domiati and Mish samples were  $3 \times 10^5/7 \times 10^3$ ,  $1 \times 10^5/4 \times 10^3$ , and  $5 \times 10^5/3 \times 10^4$  as reported in Table 3.

**Table 5.** Biochemical, serological, molecular identification, and incidence of virulence genes of *E. coli* isolated from examined samples using PCR.

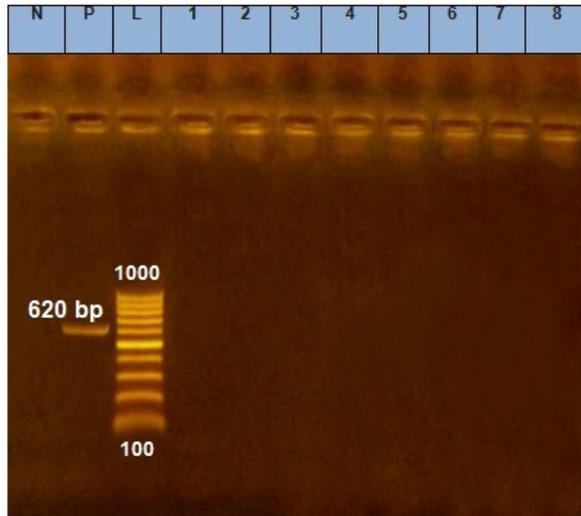
Types of samples	Samples containing <i>E. coli</i> strains identified biochemically		Strains identified serologically		Strains identified by <i>phoA</i> gene	Strains harbor <i>Stx1</i> gene	Strains harbor <i>Tsh</i> gene	Strains harbor <i>Iss</i> gene	Strains harbor <i>fimH</i> gene	
	No.	%	No.	serotype						
				No.						type
Ras cheese	4	8	4	3	O125			4	4	
				1	O18					
Domiati cheese	2	4	2	2	O125	Nil	Nil	2	2	
Mish	2	4	2	2	O114			2	2	



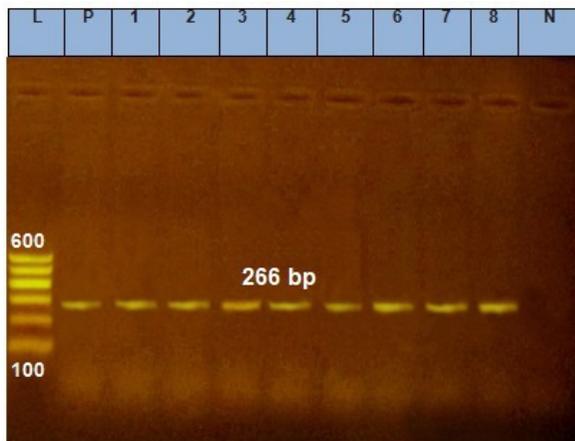
**Fig. 1.** Agarose gel electrophoresis of the PCR product of *phoA* gene for DNA extracted from analyzed *E. coli* isolates. Lane L: ladder; Lane P: positive control. Lanes 1–8: showing positive *E. coli* strains at 720 bp.



**Fig. 2.** Agarose gel electrophoresis of *E. coli* *stx1* virulence gene. Lane L: ladder; Lane P: positive control. Lanes 1–8: showing negative strains at 614 bp.



**Fig. 3.** Agarose gel electrophoresis of *E. coli tsh* virulence gene. Lane L: ladder; Lane P: positive control. Lanes 1–8: showing negative strains at 620 bp.

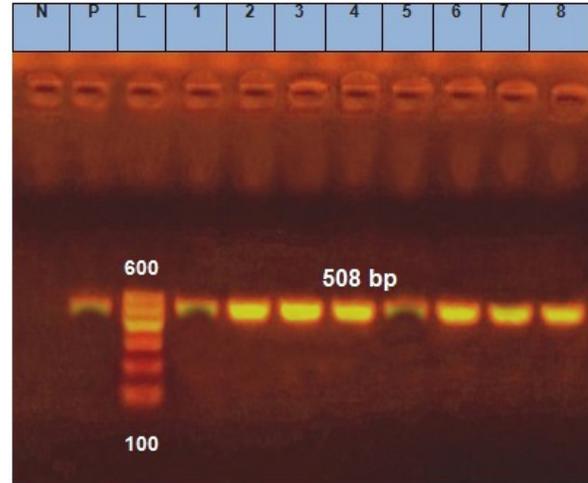


**Fig. 4.** Agarose gel electrophoresis of *E. coli iss* virulence gene. Lane L: ladder; Lane P: positive control. Lanes 1–8: showing positive strains at 266 bp.

## Discussion

### Chemical analysis

The chemical parameters, including moisture, salt, and acidity, are shown in Table 2; for Ras cheese samples, nearly similar results were obtained by Ahmed (2016) and El-Refaay *et al.* (2016) concerning salt and acidity, while higher results were recorded by Awad *et al.* (2003), Osman *et al.* (2011), and Dahmash *et al.* (2019) for salt and moisture. For Domiati cheese samples, nearly similar results for salt were obtained by Aly *et al.* (2007) and El-Baradei *et al.* (2007), higher results recorded by El-kholy *et al.* (2014), El-Zahar (2014), and El-Refaay *et al.* (2016) for salt and acidity. The



**Fig. 5.** Agarose gel electrophoresis of *E. coli fimH* virulence gene. Lane L: ladder; Lane P: positive control. Lanes 1–8: showing positive strains at 508 bp.

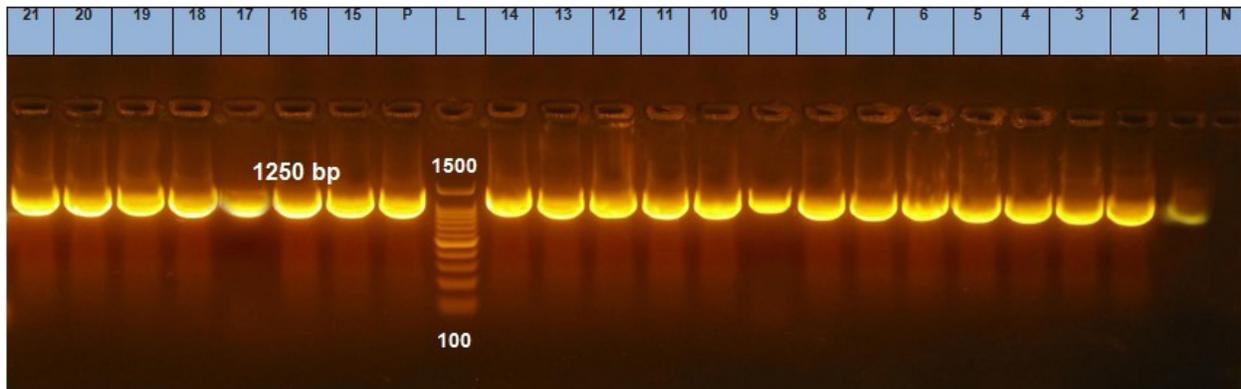
results for the Mish samples were in agreement with El-Zahar (2014) and El-Refaay *et al.* (2016). The apparent variation among the examined parameters in different examined samples could be attributed to variances in composition, properties of raw milk, and biodiversity of the organisms in each product, as well as other ingredients employed in the manufacture of traditional raw products, especially with lack of Egyptian standards for such products (Aly *et al.*, 2007).

### Microbiological examination

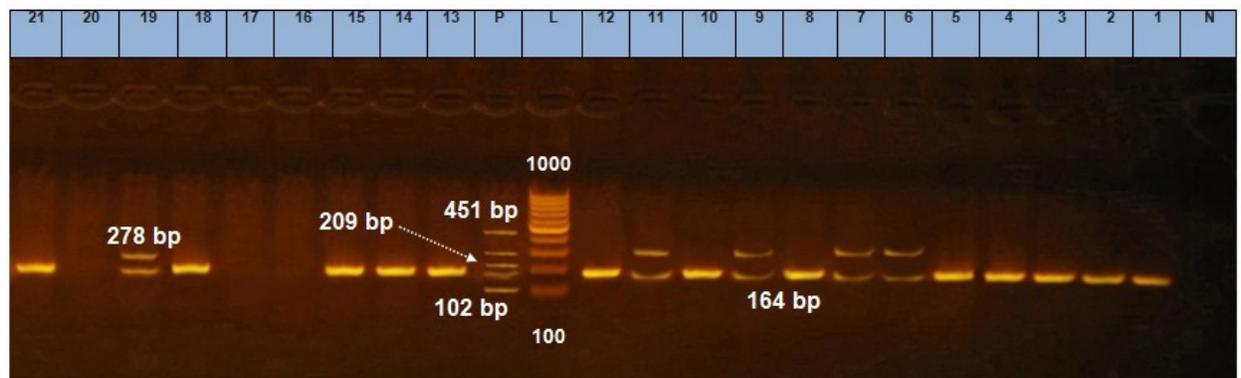
Total bacterial, presumptive coliform, yeast and mold counts were considered the most significant indices for the microbial quality (APHA (American Public Health Association), 2004). Nearly similar results of TBC for Ras cheese samples were obtained by Awad *et al.* (2003), El-Zahar (2014), and El-Leboudy *et al.* (2014), while lower results were found by Osman *et al.* (2011) and Abd El-Halem *et al.* (2019). The results of TBC for Domiati cheese samples were in agreement with those obtained by Aly *et al.* (2007), Sayed *et al.* (2011), and Ibrahim *et al.* (2015a), and higher results were obtained by El Sayed *et al.* (2011), while lower findings were obtained by El-kholy *et al.* (2014) and Ibrahim *et al.* (2015b). Nearly similar TBC results for Mish samples were reported by Zaki and Shokry (1988) and El-Zahar (2014). TBC is not directly related to the presence of pathogens or toxins, but it may be a convenient indicator of poor sanitation, defects in process control systems, and/or contaminated raw ingredients (APHA (American Public Health Association), 2004; Silva *et al.*, 2019). There was a negative correlation between TBC, salt, and acidity in the all examined samples, but was not significant as not all but most microorganisms were suppressed by salt and acidity content (Aly *et al.*, 2007). Nevertheless, there was a positive significant correlation between TBC and moisture in Domiati

**Table 6.** Biochemical, molecular identification, and incidence of enterotoxin genes of *S. aureus* isolated from examined samples using multiplex PCR.

Types of samples	Samples harbor coagulase positive <i>S. aureus</i>		Strains identified by 23S rRNA	Samples harbor enterotoxigenic strains	Type of SEs				
	No.	%			No.	No.	No.	No.	No.
Ras cheese	13	26	2	2	Nil	2	Nil	1	Nil
Domiaty cheese	18	36	13	10		10		3	1
Mish	9	18	6	6		6		Nil	Nil



**Fig. 6.** Agarose gel electrophoresis of the PCR product of 23S rRNA gene for DNA extracted from analyzed *S. aureus* isolates. Lane L: ladder; Lane P: positive control. Lanes 1–21: showing positive *S. aureus* strains at 1,250 bp.



**Fig. 7.** Agarose gel electrophoresis showing multiplex PCR amplification products for *S. aureus* enterotoxin genes. Lanes L, ladder lane P: positive control. Staphylococcal enterotoxin A (*sea*) positive isolates at 102 bp. Staphylococcal enterotoxin D (*sed*) positive isolates at 278 bp. Staphylococcal enterotoxin E (*see*) positive isolates at 209 bp. Staphylococcal enterotoxin B (*seb*) positive isolates at 164 bp. Staphylococcal enterotoxin C (*sec*) positive isolates at 451 bp.

and Mish cheese samples (Table 4). The relatively high moisture allows the growth of different types of desirable and undesirable microorganisms that may be introduced through raw milk used or during the processing steps (Aly *et al.*, 2007).

The presence of coliforms in cheese is objectionable as it reflects unsanitary conditions during production, handling, storage, and even distribution (Sayed *et al.*, 2011). Its existence may constitute a biological hazard

since coliforms are implicated in many reported cases of food poisoning. Moreover, coliforms possess an economic significance as products contaminated with coliforms will be of inferior quality and unmarketable (Martin *et al.*, 2016). Nearly similar results of coliforms for Ras cheese were obtained by El-Zahar (2014), lower results were obtained by Osman *et al.* (2011), Awad *et al.* (2003), and Abd El-Halem *et al.* (2019), while higher results recorded by Abd elsalam

(2014) and Ahmed (2016). For Domiati cheese samples, higher findings were introduced by Ibrahim *et al.* (2015b) and Abd Allah *et al.* (2017), while lower results were recorded by Sayed *et al.* (2011). For Mish samples, higher results were recorded by El-Zahar (2014). There was a positive significant correlation between coliform count and moisture in Ras cheese samples (Table 4). The comparatively low incidence of coliforms in Ras cheese samples may be related to low moisture content and consequently low water activity that will influence microbial growth (Trmčić *et al.*, 2016). Salt content had a significant negative correlation with coliforms in all examined samples as added salt not only enhances flavor but it also acts as a preservative; the anti-microbial activity of salt is owed to its ability to decrease water activity in food by damaging the microbial cell membrane (Ayyash and Shah, 2011). Concerning *E. coli* incidence rate, higher results for Ras cheese samples were obtained by Omarak *et al.* (2016) and Abd El-Halem *et al.* (2019). Nearly similar results in Domiati cheese samples were recorded by Sayed *et al.* (2011), while El-kholy *et al.* (2014) and Ibrahim *et al.* (2015b) obtained higher results. For Mish samples, higher results obtained by Dawoud *et al.* (2018). All isolated *E. coli* serotypes (O125, O18, and O114) belonging to enteropathogenic *E. coli* mainly infect infants younger than 2 years. Acute watery diarrhea, vomiting, and low-grade fever are the most detected symptoms. *E. coli* O18 also results in extra intestinal infections as neonatal meningitis, septicemia, pyelonephritis, and pneumonia (Stenutz *et al.*, 2006; Dedeić-Ljubović *et al.*, 2009; Ewida and Hussein, 2019). The molecular characterization of *E. coli* was carried out using *phoA* gene (alkaline phosphatase) as a target gene for detecting *E. coli* (Thong *et al.*, 2011) and all *E. coli* strains were positive for (*phoA*) gene. *E. coli* bacteria possesses multiple virulence genes from which four genes were detected (*stx1*, *tsh*, *iss*, and *fimH*); all detected isolates were positive for both (*iss*) and (*fimH*) genes. The virulence gene *iss* encodes a protein associated with increased serum survival of *E. coli* in humans and is considered as a protective factor for the pathogen against phagocytosis. *E. coli* acquires this gene to have the ability to cause extra intestinal infections, such as neonatal meningitis and sepsis (Sarowska *et al.*, 2019). The adhesion criteria of *E. coli* are related to *fimH* virulence gene which is important for both normal ecosystem and pathogenicity of *E. coli*. Although 95% of *E. coli* isolates show expression for type 1 fimbriae, the gene is not essential for the presence of *E. coli* in the colon. However, it helps *E. coli* to transitory colonize in the oropharyngeal portal which is significant for normal fecal/oral transmission of *E. coli* among hosts, as well as it plays an important role in the incidence of urinary tract infections caused by *E. coli* (Sokurenko *et al.*, 1998; Sarowska *et al.*, 2019).

Staphylococci are widespread microorganisms that are present everywhere. Staphylococci outbreaks are usually caused by food handlers with bad personal hygiene (EL-Kholy *et al.*, 2014). Our results (Table 4) showed that there was a significant positive correlation between moisture and staphylococci count and a significant negative correlation between it and acidity in Domiati cheese samples. While staphylococci count significantly decreases with increase in salt and acidity in Mish samples, the growth of staphylococci relies mainly on many parameters, such as temperature, pH, salt, water activity, and oxidation reduction potential; their growth can be decreased by the integration of two or more parameters (Bellio *et al.*, 2019). *Staphylococcus aureus* is incriminated in 9,000 deaths every year; it is regarded as the third most serious pathogen causing foodborne illnesses worldwide (Ahmed *et al.*, 2019). The presence of *S. aureus* in dairy products is regarded as a serious public health hazard as the organism may lose its viability during manufacturing processes but its preformed heat stable enterotoxin will still present and induce vomiting and diarrhea within 1–6 hours. However, food poisoning is not only the main health problem concerning *S. aureus*, but it also causes toxic shock syndrome, pneumonia, post-operative wound infection, and skin infections (FDA (Food and Drug Administration), 2012; EL Malt, 2013; Bellio *et al.*, 2019). Large numbers of extracellular proteins and toxins are produced by *S. aureus*. The most important toxins are Staphylococcal enterotoxins (SEs), which are SEA, SEB, SEC, SED, and SEE, that cause 95% of staphylococcal food poisoning cases, while only 5% of outbreaks occurred by newly described SEs (SEG-SEI, SEIJ, SEIQ, SER-SET, and SEIU-SEIV) and TSST-1. SEs are soluble in water, stable against most proteolytic enzymes (pepsin and trypsin), especially SEB, and heat-resistant even after boiling for 30 minutes (Bhunja, 2008; Ahmed *et al.*, 2019). The detection of the five classical enterotoxin genes of *S. aureus* (Fig. 7) revealed that Ras cheese strains harbor the *seb* and *sed* genes, while Domiati cheese strains were harboring *seb*, *sed*, and *see* genes. Moreover, Mish samples *S. aureus* strains harbor the *seb* gene. In Egypt, Abolghait *et al.* (2020) recently reported similar results that methicillin-resistant *S. aureus* isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. Production of enterotoxins by *S. aureus* is influenced by a combination of several factors, such as moisture, water activity, salt, acidity, and temperature as well as microbial competition (FDA (Food and Drug Administration), 2012; Bellio *et al.*, 2019). The low pH and the high nutritional profile of most cheeses are favorable for the growth of yeasts and molds. Their presence in cheese not only influences the sensory characteristics and decreases the grading of cheese but it also possesses a potential hazard. Some of them have the ability to produce several toxins, such as

aflatoxins, and others have the ability to cause human and animal pathogenicity (Ledenbach and Marshall, 2009; ELbagory et al., 2014; El-Fadaly et al., 2015). Nearly similar results of yeast and mold counts for Ras samples were obtained by El-Zahar (2014) and El-Fadaly et al. (2015), and lower counts were recorded by ELbagory et al. (2014). Nearly similar results in Domiati cheese were obtained by Sayed et al. (2011), higher results were obtained by Aly et al. (2007), and lower results were recorded by Ibrahim et al. (2015b). The results of Mish samples are well in line with those reported by El-Zahar (2014) and El-Refaay et al. (2016). In Mish samples, a significant positive correlation was found between yeast and moisture, as well as a significant negative correlation with acidity. The higher moisture content of the product, the more susceptibility for spoilage, off flavors, and over ripening, which result in more breakdown of acids, sugars, proteins, and lipids (Morales et al., 2003; Aly et al., 2007). Corresponding to the relatively high counts of yeasts and molds in the examined samples, using raw milk, poor cleaning practices, and inadequate ripening and storage conditions are the main causes (D'Amico, 2014).

### Conclusion

Enterotoxigenic *S. aureus* harboring *seb* gene and enteropathogenic *E. coli* (serogroups O18, O114, and O125) were frequently isolated from soft and hard artisanal cheeses in Egypt. Therefore, strict hygienic measures should be applied during their manufacture, handling, and distribution. Also, more awareness training programs for producers of dairy products should be conducted about how to produce safe products through the implementation of the recent food safety management systems as good manufacturing practices as well as hazard analysis and critical control points in production sites. Also, consumers should be aware of the correct handling and storage methods in order to maintain product safety.

### Authors' contribution

Ashraf Ahmed Moawad and Eman Fathi Abdel-Latif designed and supervised the study as well as reviewed and approved the final version of the submitted manuscript. Ola Wagih Hegab performed the experiments, analyzed, interpreted the data, and drafted the manuscript.

### Conflict of interest

All authors declare that they have no conflict of interest.

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