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Microbiological evaluation of some Egyptian fermented dairy products

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ABSTRACT: This study was carried out to evaluate the microbiological quality of plain and fruit yoghurt and rayeb. Sixty samples of yoghurt (30 plain & 30 fruit) and 60 samples of rayeb (30 plain & 30 fruit) were randomly collected from different supermarkets in Kafr El-Sheikh city. The obtained results revealed that the pH values of plain and fruit yoghurt were significantly higher than those of plain and fruit rayeb. *Staphylococcus aureus* counts were $4.33 \times 10^2 \pm 0.42 \times 10^2$ and $4.0 \times 10^2 \pm 0.57 \times 10^2$ cfu/g, in fruit yoghurt and plain rayeb, respectively. Only two samples of plain yoghurt and fruit rayeb contained *S. aureus* with counts of $9.0 \times 10^2 \pm 1.0 \times 10^2$ and $1.1 \times 10^3 \pm 0.1 \times 10^3$ cfu/g, respectively. Coliforms were only detected in plain and fruit yoghurt with counts of $8.82 \times 10^4 \pm 0.81 \times 10^4$ and $5.4 \times 10 \pm 1.04 \times 10$ cfu/g, respectively. *E. coli* was isolated from plain and fruit yoghurt with incidence rates of 73.3 and 6.7%, respectively. All *E. coli* isolates from plain yoghurt were serologically identified as, O91: H21, O26: H11, O121: H7, O163: H2, O114: H4, O128: H2, O111: H2, and O86. Fruit yoghurt had only O₁₁₁:H₂. Yeast and molds were also found in the four dairy products with high frequencies for *Penicillium species*. The two virulence genes, enterotoxin gene A and Shiga toxins 2, were detected in three isolates of *S. aureus* and *E. coli* by multiplex PCR. With these results, we could conclude that yoghurt and rayeb samples collected from Kafr El-Sheikh city supermarkets create a health threat to consumers. Therefore, the application of hygienic measures and sanitary practices is necessary to produce high-quality fermented products to assure consumer's safety.

Keywords: Fermented dairy products, *S. aureus*, Coliforms, *E. coli*, yeast, mold.

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INTRODUCTION

Fermented milk products are a crucial supplement to people's daily diet and supply necessary components for growth and better health (Uccello et al., 2012). Among these dairy products, yoghurt comes on the top for its higher nutritive value as it is rich in fat, lactose, protein, and, calcium. It is easily digested and has an inhibitory effect against harmful bacteria in the intestine. These bacteria can harm the intestines, thereby reducing intestinal motility, auto-intoxication, and, inflammation (Khan et al., 2008). Rayeb is a curdled skim milk made in Egypt. It can be prepared by heating fresh cow milk in a hot oven for 2 hrs and then the milk was left at room temperature for a period of time ranged from 1 to 4 days based on the temperature until the milk curdles formed at the bottom underneath the fat (Benkerroum and Tamine 2004). As a fermented dairy product, Rayeb is rich in vital elements with a higher nutritional value (Samet-Bali and Attia 2012). Both yoghurt and rayeb could be provided either plain (without fruits) or with fruits.

These dairy products could be contaminated with various microorganisms, such as bacteria, molds, and yeasts during manufacturing, handling, and distribution. In such a case, people consumed these contaminated dairy products would suffer from food poisoning. People also do not purchase spoiled products, thereby leading to economic losses for dairy manufacturers (Weerasekara et al., 2010). Enterotoxigenic *S. aureus* pose a safety hazard for consumers, and such existence in dairy products could be used for risk assessment of such products (Zouharova and Rysanek, 2008). *S. aureus* is one of the most prevalent food poisoning bacteria. The presence of this bacterium in dairy products indicates the unsanitary handling of the products at an improper temperature and time conditions (Collins et al., 2010; Huang et al., 2001). Among the five known *S. aureus* enterotoxins (SEA, SEB, SEC, SED, and SEE), SEA plays a key role in food-poisoning outbreaks world-wide (Argudin et al., 2010). These SEs are heat stable (remain active even after the death of *S. aureus* by cooking heat) and withstand the effect of digestive enzymes (Presscott et al., 2012).

The presence of any gram-negative bacteria such as coliforms group in commercially produced yogurt indicates post-pasteurization contamination or pasteurization failure (Hervert et al., 2017). According to the National Agency of Food and Drug Administration Control (NAFDAC), there can be neither *E.*

coli nor coliforms in each 100 ml yoghurt sample (Mbaeyi-Nwaoha et al., 2012). Coliforms are regularly utilized as an indicator of milk products quality (Yabaya and Idris, 2012). The presence of *E. coli* in dairy products indicates fecal contamination of these products (Singh and Prakash, 2008). Diarrhoeagenic *E. coli* (DEC) are the main cause of diarrhea and classified depending on their virulence factors into enterohaemorrhagic *E. coli* (EHEC), which is a subgroup of shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), and necrotoxic *E. coli* (NTEC) (Vilchez et al., 2009). Pathogenic *E. coli*, especially STEC, is also responsible for foodborne diseases which can cause bloody diarrhea, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura (Altalhi and Hassan, 2009; Kuyucuoglu et al., 2012; Paton and Paton, 1998). The main route of *E. coli* infection is the oral route through the consumption of contaminated food and dairy products (Pearce et al., 2006).

Yeasts and molds also participate in spoilage of yoghurts because acidic pH of yoghurts can create an appropriate environment for yeasts and molds growth (Fleet and Mian, 1987). Therefore, the existence of yeasts and/or molds in yogurt indicates unsanitary procedures in production or packaging (Arnott et al., 1974). Yogurts supplemented with sugar is more prone to yeast growth (Lourens-Hattingh and Viljoen, 2001).

This study aimed to assess the microbiological quality of yoghurt and rayeb in Kafr El-Sheikh city through the identification of *S. aureus*, coliform bacteria, enteropathogenic *E. coli*, yeast and molds, in addition to detection of virulence genes of *S. aureus* and *E. coli* isolates.

MATERIAL AND METHODS

All experiments were carried out in microbiology laboratories in Animal Health Research Institute and Faculty of Veterinary Medicine, Kafrelsheikh University.

Samples collection

Sixty samples of yoghurt (30 plain & 30 fruit) and 60 samples of Rayeb (30 plain & 30 fruit) were purchased from different supermarkets in Kafr El-Sheikh province. All collected samples were within the expiry date printed on the label. After purchasing, they

were placed in an insulated icebox and transported quickly to the Lab for further examination.

Determination of pH

The determination of pH of samples was performed using electrical pH meter (Bye model 6020, USA) according to the method described by Omokaro and Telema (2014).

Microbiological examination

Serial dilutions were prepared from all samples according to APHA, 2004. *S. aureus* was isolated using Baird-Parker agar medium and enrichment culture medium Giolitti-Cantonias previously described (Singh and Prakash, 2008). Pure *S. aureus* culture was characterized by the following biochemical tests: coagulase, catalase, oxidase, mannitol, hemolysis, and fermentation of glucose, lactose and sucrose (MacFaddin, 2000). Coliforms were counted using 3 tubes MPN technique on Lauryl Tryptose broth (Verma et al., 1999). *E. coli* were isolated using Eosin Methylene Blue agar (EMB, Oxoid, England) following the protocol of De Boer and Heuvelink, (2000). The isolated *E. coli* were serotyped using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) as previously detailed (Kok et al. 1996). Yeast and mold were counted using Sabouraud Dextrose Agar medium supplemented with 0.05 mg/ml chloramphenicol (Bailey and Scott, 1998). Identification of isolated mold species was done as previously described (Raper and Fennel 1965; Raper and Thom 1969; Samson 1979; Samson et al. 1976; Zycha et al. 1969).

Multiplex polymerase chain reaction (PCR)

Bacterial genomic DNA was extracted from *S. aureus* and *E. coli* isolated from plain yoghurt using QIAamp DNA mini kit following the manufacturer's instruction and as previously described (Allam et al., 2019). Multiplex PCR was used to detect the presence of virulence genes of *S. aureus* and *E. coli* using specific primers (Table 1). A total PCR reaction volume of 30 µl was used. This mixture included 15 µl 2x Master mix (Emerald Amp GT), 5 µl DNA, 1 µl from each forward primer and reverse primer (20 pmol), 8 µl nuclease free water. The thermal cycling conditions were: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 40 sec for *stx1* and *stx2* of *E. coli* and 57°C 40 sec for *S. aureus* enterotoxin genes, extension at 72°C for 40 sec, followed by a final extension at 72°C for 10 min. A volume of 20 µl of each PCR product was used in gel electrophoresis. A 100bp DNA ladder was used as a marker for PCR products. This gel contained 1.5 % agarose gel and 0.5 µg/ml ethidium bromide (for staining). The gel was photographed by gel documentation equipped with UV transilluminator.

Statistical analysis

Data were presented as a mean ± standard error of the mean (SEM) and significance was set at $P < 0.05$. Statistical analysis was achieved using either One-way ANOVA or unpaired t-test. The post-hoc test, Tukey's Honestly Significant Difference was used to determine the difference between the groups using GraphPad Prism 7.

Table.1. Oligonucleotide primers sequences

	Gene	Sequence	Amplified product (bp)	References
S. aureus	Sea	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102	Mehrotra et al., 2000
	Seb	GTATGGTGGTGTAAGTACTGAGC CCAAATAGTGACGAGTTAGG	164	
	Sec	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	451	
	Sed	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278	
	See	AGGTTTTTTCACAGGTCATCC CTTTTTTTCTTCGGTCAATC	209	
E. coli	Stx 1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614	Dipineto et al., 2006
	Stx 2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	779	

RESULTS AND DISCUSSION

The obtained results showed that the pH values of plain and fruit yoghurt were significantly ($P < 0.001$) higher than those of plain and fruit rayeb (Table 2). However, no significant difference was noticed between either plain and fruit yoghurt or plain and fruit rayeb ($P > 0.05$). Similar pH values were obtained by Osman (2015) for plain and strawberry fruit yoghurt (4.77 ± 0.03 and 4.37 ± 0.02 , respectively) and rayeb samples (4.47 ± 0.03). However, El-Ansary (2014) reported lower pH values for yoghurt samples (4.06 ± 0.068). Higher pH of yoghurt might be due to the absence of accurate system of culture dosage, which mostly influences yoghurt acidity (Abrar et al., 2009).

Table 2. The pH values in examined samples

Samples	pH values
Plain yogurt	4.51 ± 0.0^5 a
Fruit yogurt	4.43 ± 0.0^4 a
Plain rayeb	4.21 ± 0.0^3 b
Fruit rayeb	4.16 ± 0.0^2 b

Data were expressed as mean \pm SEM. Means carrying different superscript letters [a (the highest value), b (the lowest value)] in the same column differed significantly at $P < 0.001$.

According to the standards of Egyptian Organization for Standardization and Quality Control fermented each 1 g of dairy products should be free from *S. aureus* (EOSQC, 2005). The microbiological analysis results revealed the presence of *S. aureus* in plain yoghurt, fruit yoghurt, plain rayeb, and fruit rayeb with incidence rates of 6.67, 20, 13.33, and 6.67%, respectively (Table 3). Samet-Bali et al. (2016) reported a similar prevalence of *S. aureus* in rayeb (13.33%). However, Atef et al., (2017), El-Ansary, (2014), and El-Leboudy et al., (2017) found a high number of *S. aureus* (25% in rayeb, 42% in yoghurt, and 85% in Rayeb, respectively). On the

other hand, AbdEllatif et al., (2016) and Bachir and Benattouche, (2013) could not isolate *S. aureus* from any examined yoghurt samples. *S. aureus* count was significantly ($P < 0.01$) higher in plain yoghurt, and fruit rayeb than in fruit yoghurt and plain rayeb (Table 3). However, no significant difference was observed between either plain yoghurt and fruit rayeb or fruit yoghurt and plain rayeb ($P > 0.05$). Higher *S. aureus* count in yoghurt ($5.5 \times 10^4 \pm 3.94 \times 10^3$ cfu/g) was reported by El-Ansary, (2014). In general, the higher prevalence of *S. aureus* in these dairy products might be due to the unhygienic practices during production and processing (Salvatierra et al., 2004). Presence of *S. aureus* in fermented dairy products indicates contamination from food handlers through hand or arm lesions caused by *S. aureus*. Therefore, dairy products could be contaminated through contact, coughing and sneezing (Hussain 2010).

It is obvious from Table 3 that coliforms were only detected in plain and fruit yoghurt with incidence rates of 100% and 13%, and counts of $8.82 \times 10^4 \pm 0.81 \times 10^4$ and $5.4 \times 10 \pm 1.04 \times 10$ cfu/g, respectively. Plain yoghurt exhibited a significantly higher coliforms count than fruit yoghurt. Lower coliforms prevalence of 58% and 40% with a count of $5.6 \times 10^4 \pm 3.68 \times 10^3$ and $5.02 \times 10^2 \pm 0.57 \times 10^2$ cfu/g, were reported in plain yoghurt by El-Ansary (2014) and Osman (2015), respectively. In contrast, El-Leboudy et al., (2017) reported that the prevalence and count of coliforms in rayeb samples was 100% and $1.65 \times 10^3 \pm 2.69 \times 10^2$ cfu/g. Egyptian Organization for Standardization and Quality Control declared that each 1 g of fermented dairy products should be free from coliforms (EOSQC, 2005). High count of coliforms in yoghurt indicates unsanitary procedures, utilizing of low quality milk, inadequate preheating, post-processing cross-contamination (El Bakri and El Zubeir, 2009; Samet-Bali et al., 2016).

Table 3. *S. aureus* and Coliforms count in examined samples

Samples	<i>S. aureus</i>		Coliforms	
	NO(%)	Count	NO(%)	Count
Plain yogurt	2(6.67)	$9.0 \times 10^2 \pm 1.0 \times 10^{2a}$	30(100)	$8.82 \times 10^4 \pm 0.81 \times 10^{4a}$
Fruit yogurt	6(20)	$4.33 \times 10^2 \pm 0.42 \times 10^{2b}$	4(13)	$5.4 \times 10 \pm 1.04 \times 10^b$
Plain rayeb	4(13.33)	$4.0 \times 10^2 \pm 0.57 \times 10^{2b}$	0	0
Fruit rayeb	2(6.67)	$10.1 \times 10^2 \pm 1.1 \times 10^{2a}$	0	0

Data were expressed as mean \pm SEM. Means carrying different superscript letter in the same column differed significantly at $P < 0.01$.

E.coli was only detected in plain and fruit yoghurt samples at prevalence rates of 73.3 and 6.7%, respectively (Table 4). This exceeded the limit set by Egyptian Organization for Standardization and Quality Control, which declared that each 1 g of fermented dairy products should be free from *E.coli* (EOSQC, 2005). The isolated strains from plain yoghurt. were serotyped to O91 : H21 (EHEC) , O26 : H11 (EHEC), O121: H7 (EHEC), O163 : H2 (EPEC), O114 : H4 (EPEC), O128: H2 (ETEC), O111: H2 (EHEC) and O86 (EPEC) at prevalence rate 12.5, 37.5, 6.3, 6.3, 12.5, 12.5, 6.3 and 6.3 %, respectively. The isolated *E.coli* from fruit yogurt were serotyped into O111: H2(EHEC) at an incidence rate of 100%. O₂₆: H₁₁ was the most frequently identified serotypes isolated from plain yoghurt and was detected with higher frequency 37.5% followed by O91: H21, O114: H4 and O128: H2 with a prevalence rate 12.5 % for each *E.*

coli O26:H11 is commonly associated with diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (Anjum et al., 2003; Bettelheim, 2003). Lower prevalence of *E.coli* (44.8%) was detected in plain yoghurt (El-Ansary, 2014). However, Atef et al., (2017) and El-Leboudy et al., (2017) reported *E.coli* number of 55% in rayeb sample and 30% in Baladi rayeb, respectively. In contrast, El-Baz, (2019) did not detect verotoxigenic *E.coli* in yoghurt. Higher number of *E.coli* in plain yogurt could be due to insufficient heating, using poor quality raw milk containing higher initial bacterial count, and unsanitary handling. The variations in *E.coli* incidence ratio and count in fermented dairy products among different studies could be attributed to the fact that the survival of *E.coli* in fermented dairy products is highly variable depending on starter cultures used, temperature of storage and pH value.

Table 4. Incidence and serotyping of *E. coli* isolated from examined samples (n=60)

Products	Plain yogurt		Fruit yogurt		Strains characterization
	No	%	No	%	
<i>E.coli</i>	22	73.3	2	6.7	
	Serotypes of isolated <i>E.coli</i>				
Serotype	No	%	No	%	
O ₉₁ : H ₂₁	4	12.5	-	-	EHEC
O ₂₆ : H ₁₁	12	37.5	-	-	EHEC
O ₁₂₁ : H ₇	2	6.3	-	-	EHEC
O ₁₆₃ : H ₂	2	6.3	-	-	EPEC
O ₁₁₄ : H ₄	4	12.5	-	-	EPEC
O ₁₂₈ : H ₂	4	12.5	-	-	ETEC
O ₁₁₁ : H ₂	2	6.3	2	100	EHEC
O ₈₆	2	6.3	-	-	EPEC

Yoghurt is a highly selective environment for the growth of yeast and mold due to its high acidity. Yeast and mold can utilize acid as a source of energy and produce alkaline products that decrease acidity and give a chance for the growth of putrefactive bacteria (Abdel Hameed, 2011). The allowed limits for yeasts and molds in fermented dairy products are less than 10 /g as stated by Egyptian standards (EOSQC, 2005). The presented data in table 5 elucidated that the yeasts were detected at incidence rates of 93.33, 26.67, 33.33, and 26.67 % with counts of $3.92 \times 10^4 \pm 0.81 \times 10^4$, $2.90 \times 10^4 \pm 1.88 \times 10^4$, $2.30 \times 10^2 \pm 0.73 \times 10^2$, and $2.75 \times 10^3 \pm 1.42 \times 10^3$ cfu/g in plain, fruit yoghurt and plain, and fruit rayeb, respectively. Yeast count was significantly higher in plain yoghurt than in other examined dairy products. Similar yeast incidence of 92.0% and count $4.2 \times 10^4 \pm 0.8 \times 10^4$ cfu/g in yoghurt was reported by AbdEllatif et al., (2016). A higher prevalence of 100% with a count of $8.37 \times 10^5 \pm 0.96 \times 10^5$ cfu/g was

recorded by Osman, (2015). Lower number (40%) but with similar count ($5.6 \times 10^4 \pm 1.6 \times 10^4$ cfu/g) was detected in plain yoghurt (Barakat, 2019). Molds were detected at incidence rates of 66.67, 80, 100, and 100% with counts of $1.74 \times 10^3 \pm 0.52 \times 10^3$, $4.25 \times 10^2 \pm 0.67 \times 10^2$, $1.95 \times 10^3 \pm 0.38 \times 10^3$, and $8.06 \times 10^2 \pm 0.82 \times 10^2$ cfu/g in plain yoghurt, fruit yoghurt, plain rayeb, and fruit rayeb, respectively (Table 5). Fruit yoghurt showed significantly lower mold count than other examined dairy products. A similar number was obtained by Osman, (2015), who reported that 100% of examined rayeb samples were contaminated with mold with an account of $4.49 \times 10^5 \pm 0.51 \times 10^5$ cfu/g. In addition, Samet-Bali et al., (2016) reported that 100% of rayeb samples were contaminated with yeast and mold but with a higher count of $1.34 \times 10^7 \pm 0.23 \times 10^7$ cfu/g. Lower mold count (28%) with a level of $1.5 \times 10^4 \pm 0.44 \times 10^4$ cfu/g was detected by Barakat, (2019). Amer, (2017) reported that 44 % of examined

yoghurt samples were contaminated with molds with a count of $3.92 \times 10^3 \pm 7.67 \times 10^2$ cfu/g. The presence of yeasts and molds in yoghurt indicates poor sanitary conditions (Oyeleke, 2009 and El-Malt et al., 2013).

Penicillium species were the most frequent (21.7%, 31.0, 23.6, and 33.3%) isolated mold species from plain, fruit yoghurt and plain, and fruit rayeb, respectively (Table 6). Aspergillus species were the second most prevalent mold in fermented dairy products. Similarly, Osman, (2015) also found that Penicillium species were the most frequent isolated mold species from examined plain yoghurt, flavored yoghurt, and rayeb samples. While, Barakat, (2019) found that Geotrichum species was the most frequent isolated mold species from yoghurt samples at an incidence rate 32.9%. Frequent isolation of Penicillium species from dairy products could be due to ability of these species to grow within wide ranges of pH (2 to 11), water activity value (0.620 to 0.995), temperature (-10 to 60 °C) and nutrient limitations (Pitt and Hocking 2009).

Results obtained from multiplex PCR revealed positive results for only enterotoxin A (*sea*) virulent gene (102 bp) in *S. aureus* isolates 1, 2, and 4 (Fig.1). Consistent with these results, Mohamed and Mazyed (2015) also detected *sea* gene in one isolate from three examined plain yoghurts. These results suggest that the PCR assay is a fast and highly sensitive technique for the determination of enterotoxins genes in *S. aureus* isolates (Anvari et al., 2008). Multiplex PCR for detection virulence genes in *E. coli* isolates revealed that only *stx2* gene (779 bp) was detected in isolates 2, 3, and 4 (Fig. 2). Our results agreed with Neven et al., (2017) who also detected *stx2* in *E. coli* isolated from plain yoghurt. Stx proteins produced by *E. coli* are one of the main causes of enteritis in humans (Tyler, et al. 2005). Virpari et al. (2013) reported that out of 80 *E. coli* isolates from milk and dairy products 12 (15 %) and 18 (22.50%) isolates were positive for *stx1* and *stx2* gene, respectively. Detection of *sea* and *stx2* does not confirm their production so RPLA technique could be used to evaluate toxin production (Vanbelkum, 2003).

Table 5. Yeast and mold count in examined samples

Samples	Yeast		Mold	
	NO(%)	Count	NO(%)	Count
Plain yogurt	82(93.33)	$3.92 \times 10^4 \pm 0.81 \times 10^4$ ^a	20(66.67)	$1.74 \times 10^3 \pm 0.52 \times 10^3$ ^a
Fruit yogurt	8(26.67)	$2.90 \times 10^4 \pm 1.88 \times 10^4$ ^b	24(80)	$4.25 \times 10^2 \pm 0.67 \times 10^2$ ^b
Plain rayeb	10(33.33)	$2.30 \times 10^2 \pm 0.73 \times 10^2$ ^c	30(100)	$1.95 \times 10^3 \pm 0.38 \times 10^3$ ^a
Fruit rayeb	8(26.67)	$2.75 \times 10^3 \pm 1.42 \times 10^3$ ^{bc}	30(100)	$8.06 \times 10^2 \pm 0.83 \times 10^2$ ^a

Data were expressed as mean \pm SEM. Means carrying different superscript letters in the same column differed significantly at $P < 0.05$.

Table 6. Incidence of identified mold species isolated from examined samples

Mold sp.	Plain yogurt		Fruit yogurt		Plain rayeb		Fruit rayeb	
	No	%	No	%	No	%	No	%
Penicillium sp.	10	21.7	18	31.0	26	23.6	20	33.3
A. niger	6	13.0	2	3.4	14	12.7	6	10.0
A. fumigatus	2	4.3	2	3.4	8	7.3	0	0
A. flavus	8	17.4	8	13.8	4	3.6	8	13.3
A. versicolor	2	4.3	0	0	2	1.8	4	6.7
A. ochraceus	2	4.3	0	0	2	1.8	2	3.3
A. terreus	0	0	8	13.8	8	7.3	0	0
A. ruber	0	0	2	3.4	0	0	0	0
A. nidulans	0	0	2	3.4	0	0	0	0
A. clavatus	0	0	0	0.0	0	0	0	0
Mucor sp.	8	17.4	2	3.4	10	9.1	6	10.0
Rhizopus sp.	2	4.3	0	0	8	7.3	0	0
Fusarium sp.	2	4.3	6	10.3	4	3.6	6	10.0
Thamnidium sp.	2	4.3	6	10.3	12	10.9	6	10.0
Trichothecium sp.	0	0	2	3.4	0	0	0	0
Cladosporium sp.	2	4.3	0	0	12	10.9	2	3.3
Total No.	46	100	58	100	110	100	60	100

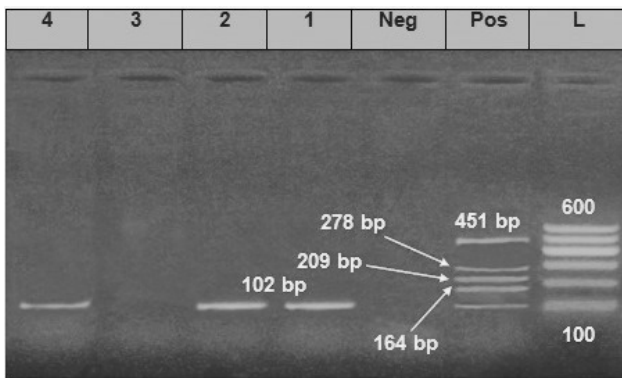


Fig.1. Agarose gel electrophoresis of multiplex PCR of *sea* (102 bp), *seb* (164 bp), *sec* (451 bp), *sed* (278 bp), and *see* (209 bp) enterotoxin genes for characterization of *S. aureus*. L: 100 bp DNA ladder; Pos: control positive for *sea*, *seb*, *sec*, *sed*, and *see* genes, Neg: Control negative; Lanes 1, 2 and 4: Positive *S. aureus* strains for *sea*, lane 3: negative for *sea*.

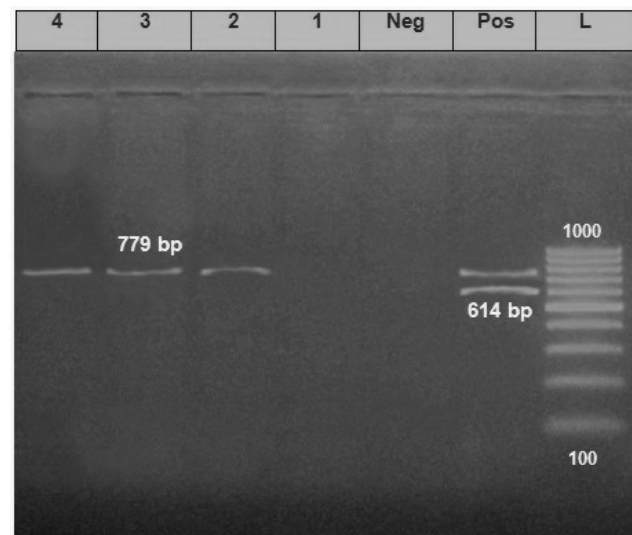


Fig.2. Agarose gel electrophoresis of multiplex PCR of *stx1* (614 bp) and *stx2* (779 bp) genes for characterization of *E. coli*. L: 100 bp DNA ladder; Pos: control positive for *stx1* and *stx2* genes, Neg: Control negative, Lanes 2, 3 and 4: Positive for *stx2*, lane 1: negative for *stx2*

CONCLUSIONS

Yoghurt and rayeb samples collected from Kafr El-Sheikh city supermarkets create a health hazards to consumers. They were contaminated, at different degrees, with *S. aureus*, coliforms, *E.coli*, yeasts, and molds. Moreover, enterotoxin A and shigatoxin 2 genes were detected in some examined isolates of *S. aureus* and *E.coli*. Presence of these micro-organisms in dairy products indicates poor sanitary procedures during the production and processing. The results of

this study warrant the need to undertake safety measures during handling, manufacturing, and distribution of fermented dairy products to avoid potential threats for consumers safety.

CONFLICT OF INTEREST

The authors certify that they have no affiliations with any organization or entity with financial or non-financial interest in the subject matter or materials discussed in this manuscript.

REFERENCES

- Abdel Hameed, K. G. 2011. Evaluation of chemical and microbiological quality of raw goat milk in Qena province Assiut Vet. Med. J. 57 (129): 131-144.
- AbdEllatif, S.S., Magdy, S.E., Ali, A. B. and Mohamed, A.B. 2016. Studies on beneficial yeasts isolated from some Egyptian dairy products. Zagazig Veterinary Journal Volume 44 (1): 75-84.
- Abrar, M., Anjum, F.R., Zahoor, T., Rahman S.U., Hussain, S., Ahmad S. 2009. Chemical and sensory characteristics of yoghurt prepared by locally isolated and commercially imported starter cultures. Milchwissenschaft. 64(4): 392-395.
- Allam SA, Mostafa NY, Kirrella GAK, Eleiwa NZ, EL-Magd MA. 2019. Molecular detection of inva and hila virulent genes in salmonella serovars isolated from fresh water fish. *Slov Vet Res*, 56:693-698.
- Altalhi, A.D., Hassan, S.A. 2009. Bacterial quality of raw milk investigated by *Escherichia coli* and isolates analysis for specific virulence-gene markers. *Food Control*, 20:913-7.
- Amer, A.R. 2017. Sanitary evaluation of milk and its products serving at general hospitals. Ph.D. Thesis, Fac. Vet. Med. Alexandria University.
- American Public Health Association (APHA) (2004): Standard Methods for the Examination of Dairy Products 17th Edition. Edited by H. Michael Wehr and Joseph H. Frank, Washington, D.C., USA.
- Anjum, M.F., Lucchini, S., Thompson, A., Hinton, J.C.D., Woodward, M.J. 2003. Comparative genomic indexing reveals the phylogenomics of *Escherichia coli* pathogens. *Infect. Immun.* 71:4674-4683.
- Anvari, S.H., Sattari, M., Forozandeh Moghadam, M., NajarPeerayeh, S.H., ImaneeFouladi, A.A. 2008. Detection of Staph. aureus enterotoxins A to D from clinical samples by PCR. *Research J. of Bio. Sci.* 3(8):826-829.
- Argudin M.A., Mendoza M.C., Rodicio M.R. 2010. Food poisoning and Staphylococcus aureus enterotoxins. *Toxins*, 2: 1751-1773.
- Arnott, D.R., Duitschaever, C.L. and Bullock, D.H. 1974. Microbiological evaluation of yoghurt produced commercially in Ontario. *J. Milk Food Technol.*, 37(1): 11-13.
- Atef, N., Mohamed I., Al-Shimaa A.S., Abdel-Rahman A-M. 2017. Molecular Characterization of Pathogenic *E. coli* and *Staphylococcus Aureus* Isolated from Some Fermented Milk Products by Using PCR. *Alexandria Journal of Veterinary Sciences*, 54: 127-134.
- Bachir, R.G., Benattouche, Z. 2013. Evaluation of the quality of steamed yogurt treated by Lavandula and Chamaemelum species essential oils. *Global Sci. Res. J.*, 1 (1), pp. 053-058.
- Bailey, W.R. and Scott, E.G. 1998. *Diagnostic Microbiology. A textbook for the Isolation and Identification of Pathogenic Microorganisms.* The C.V. Mosby Company Saint Louis.
- Barakat, S.M. 2019. Mycological evaluation of serving some dairy products with special reference to aflatoxin production in student hostel at faculty of vet. Med. Alexandria University, Edfina. M. V. Sc. Thesis, Fac.

- Vet. Med. Alexandria University.
- Benkerroum, M.N. and Tamine, A.Y. (2004): Technology transfer of Moroccan traditional dairy products (Lben, jben and smen) to small industrial scale. *Food Microbiology* 21: 399-413.
- Bettelheim, K.A. 2003. Non-O157 Verotoxin-producing *Escherichia coli*: a problem, paradox, and paradigm. *Exp. Biol. Med.* 228:333-344.
- Collins, N., Ateba, M.M., Modisane, S., Moneoang, C.C., Bezuidenhout, C. 2010. Antibiotic Resistant *S. aureus* Isolated from Milk in the Mafikeng Area, North West Province, South Africa. *South African Journal of Science*, 106:1-6.
- De Boer, E., and Heuvelink, A.E. 2000. Methods for the detection and isolation of Shiga toxin producing *Escherichia coli*. *J. Appl. Microbiol.* 88,133-43.
- Dipineto, L., Santaniello, A., Fontanella, M., Lagos, K., Fioretti, A. and Menna, L.F. 2006. Presence of Shiga toxin-producing *Escherichia coli* O157:H7 in living layer hens. *Letters in Applied Microbiology* 43 (2006) 293-295.
- El-Ansary, M.A. 2014. Assessment of Microbiological Quality of Yoghurt Sold in El-Behera Governorate. *Alexandria Journal of Veterinary Sciences*, 43: 52-57.
- El-Baz, A.H. 2019. Prevalence, Molecular Characterization and Antimicrobial Resistance of Vero Toxigenic *E.coli* in Fresh Soft Cheese, Ice Cream and Yoghurt in Mansoura City. *Alexandria Journal of Veterinary Sciences*, 62(1): 38-46.
- El-Leboudy, A.A., Amer A.A., El-Ansary M.A., El Asuoty, M.S., Marwa, R.A. 2017. Evaluation of some fermented milks sold in Alexandria city. *Assiut Vet. Med. J.* 63 (154):59-66.
- Fleet, G.H. and Mian, M. 1987. The occurrence and growth of yeasts in dairy products. *International Journal of Food Microbiology*, 4(2): 145-155.
- EOSQC (2005) Egyptian Organization for Standardization and Quality Control. Egyptian Standards (E.S.): "Domiat cheese 1008-3/ Kareish cheese 1008-4/ Ice cream 1185-1/ Yoghurt 1633".
- Heaton J.C. and Jones, K., 2008. Microbial contamination of fruit, vegetables, and the behavior of enteropathogens in the phyllosphere: a review. *J. Appl. Microbiol.*, 104 (3): 613-626.
- Hervet, C.J., Martin, N. H., Boor, K.J., and Wiedmann, M. 2017. Survival and detection of coliforms, Enterobacteriaceae, and gram-negative bacteria in Greek yogurt. *J. Dairy Sci.* 100:950-960
- Huang, S., Weng, Y., Chiou, R. 2001. Survival of *Staphylococcus aureus* and *Escherichia coli* as Affected by Ethanol and NaCl. *Journal of Food Protection*, 64:546-550.
- Hussain, K.I.S. 2010. Microorganisms involved in (Roub) traditional fermented milk in Sudan. M. Sc. Thesis, University of Khartoum, Sudan.
- Kavas, G., Kinik, O., Uysal, H., Kilic, S., Celikel, N. 2006. Characterization of yeasts Isolated from artisanal Turkish dairy products. *Int. J. Dairy Sci.*, 1: 44-50.
- Khan, K.R., Khan, S.U., Anwar, M.A., Bhadar, F.S. 2008. Physical and chemical quality appraisal of commercial yoghurt brands sold at Lahore. *ARPN. Journal of agricultural and biological science* 3(3):14-24.
- Kok, T., Worswich, D., Gowans, E. 1996. Some serological techniques for microbial and viral infections. In *Practical Medical Microbiology* (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill Livingstone, UK.
- Kuyucuoglu, Y., Kenar, B., Konak, S., Gurler, Z., & Acaroz, U. (2012). Antibacterial Resistance of Commensal *E-coli* and *E-coli* O157: H7 Strains Isolated from Cattle and Calves Faeces Samples. *Journal of Animal and Veterinary Advances*, 11(1), 52-55.
- Lourens-Hattingh, A. and Viljoen, B. 2001. Growth and survival of a probiotic yeast in dairy products. *Food Research International*, 34(9): 791-796.
- MacFaddin J.F. 2000. *Biochemical Tests for Identification of Medical Bacteria*, 3rd ed. Philadelphia: Lippincott Williams and Wilkins, 2000.
- Mbaeyi-Nwaoha, I.E. and Egbuche, N.I., 2012. Microbiological evaluation of sachet water and street vended yoghurt and Zobo drinks sold in Nsukka Metropolis. *Int. J. Biol. Chem. Sci.*, 6(4): 1703-1717.
- Mehrotra, M.; WANG, G. and Johnson, W.M. 2000. Multiplex PCR for Detection of Genes for *Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. *JOURNAL OF CLINICAL MICROBIOLOGY*. Vol. 38, No. 3.
- Mohamed Eman, F. and MazyedEbtesam, M. 2015. Incidence of *Staphylococcus aureus* and its enterotoxins in yoghurt Benha Veterinary Medical Journal, 28 (2):107-114.
- Neven A., Mohamed I., Al-Shimaa A. S., Abdel-Rahman Abdel-Mageed 2017. Molecular Characterization of Pathogenic *E. Coli* and *Staphylococcus Aureus* Isolated from Some Fermented Milk Products by Using PCR. *Alexandria Journal of Veterinary Sciences*, 54: 127-134.
- Omokaro, O. and Telema, B.B. 2014. Microorganisms associated with street vended yoghurt in mile 1 Diobu area of Port Harcourt, Nigeria. *e-Journal of Science & Technology (e-JST)*, 5(9):179-186.
- Osman, O.M.M. 2015. Quality evaluation of some fermented milk sold at local market. Ph.D. thesis, Fac. Vet. Med. Alexandria University, Egypt.
- Oyeleke, S.B. 2009. Microbial assessment of some commercially prepared yogurt retailed in Minna, Niger State. *Afr. J. Microbiol. Res.* 3: 245-248.
- Paton JC, Paton AW. 1998. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clin. Microbiol. Rev.* 11:450-479.
- Pearce MC, Evans J, McKendrick IJ, Smith AW, Knight HI, Mellor DJ, Woolhouse MEJ, Gunn GJ, Low JC. 2006. Prevalence and virulence factors of *Escherichia coli* serogroups O26, O103, O111, and O145 shed by cattle in Scotland. *Appl. Environ. Microbiol.* 72:653-659.
- Pitt, J.I. and Hocking, A.D. 2009. *Fungi and Food spoilage* (3rd Ed.). Springer Science + Business Media, LLC, 233 Spring Street, New York, NY 10013, USA.
- Presscott, L.M., Harley, J.P. and Klein, D.A. 2012. *Textbook of Microbiology*. Brown Publishers. 5th ed: 441-442.
- Raper, K.B. and Fennell, D.I. 1965. *The genus Aspergillus*. 1st Ed. Williams and Wilkins Company, Baltimore.
- Raper, K.B. and Thom, C. 1969. *A manual of the penicillia*, Williams and Wilkins Company, Baltimor.
- Salvatierra, M., Molina, A., GamboaMdel, M., Arias, M. 2004. Evaluation of the Effect of Probiotic Cultures on Two Different Yoghurt Brands over a Known Population of *Staphylococcus aureus* and the Production of Thermo-nuclease. *Archivos Latinoamericanos de Nutrición*, 54:298-302.
- Sambrook, J., Fritsch, E.F., and Maniatis 1989. *Molecular cloning. A laboratory manual*. Vol 1., Cold spring Harbor Laboratory press, New York.
- Samet-Bali, O. and Attia, H. (2012): Characterization of typical Tunisian fermented milk, rayeb. *African Journal of Biotechnology* 11(25): 6744-6749.
- Samet-Bali, O., Felfoul, I., Lajnaf, R., Attia, H. and Ayadi, M. A. (2016): Hygienic quality of "Rayeb", a traditional Tunisian fermented cow's milk. *International Food Research Journal* 23(1): 366-369.
- Samson, R.A. 1979. A complication of Aspergilli. *Studies in Mycology* 3: 198-202
- Samson, R.A., Stalk, A. and Hadlok, R. 1976. Revision on the subsection subsection fasciculata of *Penicillium* and some allied species. *Studies in Mycology* 2: 75-78.
- Singh, P. and Prakash, A. 2008. Isolation of *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* from milk products sold under market conditions at Agra Region. *Acta Agri. Slov.*, 92(1): 83-88.
- Tyler, J.S.; Livny, J.; Friedman, D.I. 2005. *Lambdoid Phages and Shiga Toxin. In Phages: Their Role in Bacterial Pathogenesis and Biotechnology*; Waldor, M.K., Friedman, D.I., Adhya, S.L., Eds.; ASM Press: Washington, DC, USA, 2005; pp. 131-164.
- Uccello, M., Malaguamera, G., Basile, F., D'agata, V., Malaguamera, M., Bertino, G., Vacante, M., Drago, F. and Biondi, A. (2012): Potential role of probiotics on colorectal cancer prevention. *BMC Surgery* 12: 45-49.
- Vanbelkum, A. 2003. Molecular diagnostics in medical microbiology: Yesterday, today and tomorrow. *Curr. Opin. Pharmacol.*, 3(5): 497-501.
- Verma, J. K., Greene, K. D., Relter, M. E., Trother, J. and Nowickiki, S. F. 1999. An outbreak of *Escherichia coli* infection following exposure to contaminated food. *JANA*: 290- 2178.
- Vilchez, S., Reyes, D., Paniagua, M., Bucardo, F., Möllby, R., Weintraub, A. 2009. Prevalence of diarrhoeagenic *Escherichia coli* in children from León, Nicaragua. *J. Med. Microbiol.*, 58:630-7.
- Virpari, P.K., Nayak, J.B., Brahmabhatt, M.N., Thaker, H.C. 2013. Study on isolation, molecular detection of virulence gene and antibiotic sensitivity pattern of *Escherichia coli* isolated from milk and milk products. *Vet world*, 41-545.
- Weerasekara, M., Karunaratne, R., Gamika, A. 2010. Assessment of quality parameters in North Central province. *Wayamba Journal of Animal Science*. 578: 26-29.
- Yabaya, A. and Idris, A. 2012. Bacteriological quality assessment of some yoghurt brands sold in kaduna metropolis. *Jorind10* (2): 35-39
- Zouharova, M. and Rysanek, D. 2008. Multiplex PCR and RPLA Identification of *Staphylococcus aureus* Strains from Bulk Tank Milk. *Zoonoses Public Health*, 55: 313-319.
- Zycha, H., Siepmann, R., Linnemann, G. 1969. *Mucorales Eine Besebreitung alter Gattungen und Artendiesserpilzgruppever lag Von. J. Carmer*.