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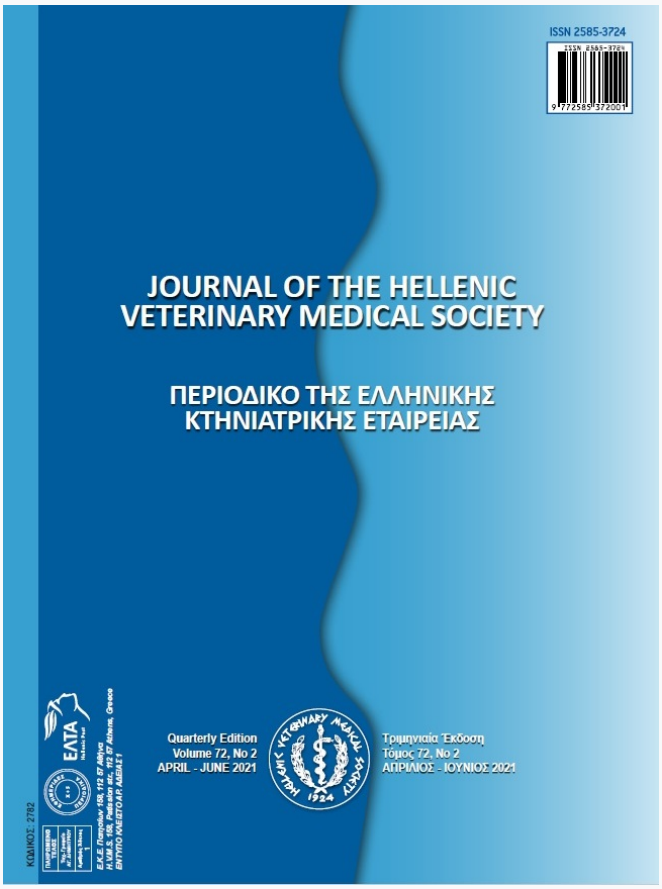


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Research article
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# Microbiological evaluation of some Egyptian fermented dairy products

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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 $4.33\times10^2 \pm 0.42\times10^2$ and $4.0\times10^2 \pm 0.57\times10^2$cfu/g, in fruit yoghurt and plain rayeb, respectively. Only two samples of plain yoghurt and fruit rayeb contained *S.aureus* with counts of $9.0\text{x } 10^2 \pm 1.0\times10^2$ and $1.1\times10^3 \pm 0.1\times10^3$cfu/g, respectively. Coliforms were only detected in plain and fruit yoghurt with counts of $8.82\times10^4 \pm 0.81\times10^4$ and $5.4\times10 \pm 1.04\times10$ 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 $O_{111}$:$H_2$. 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 practicesis 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, autointoxication, 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, Rayebis 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 rayebin 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 iceboxand 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), 5µl DNA, 1 µl from each forward primer and reverse primer (20 pmol), 8µl 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 20µl 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<br>CGGCACTTTTTTCTCTTCGG | 102 | Mehrotra et al., 2000 |
| | Seb | GTATGGTGGTGTAACTGAGC<br>CCAAATAGTGACGAGTTAGG | 164 | |
| | Sec | AGATGAAGTAGTTGATGTGTATGG<br>CACACTTTTAGAATCAACCG | 451 | |
| | Sed | CCAATAATAGGAGAAAATAAAAG<br>ATTGGTATTTTTTTTCGTTC | 278 | |
| | See | AGGTTTTTTCACAGGTCATCC<br>CTTTTTTTTCTTCGGTCAATC | 209 | |
| E. coli | Stx 1 | ACACTGGATGATCTCAGTGG<br>CTGAATCCCCCTCCATTATG | 614 | Dipineto et al., 2006 |
| | Stx 2 | CCATGACAACGGACAGCAGTT<br>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, whichmostly influences yoghurt acidity (Abrar et al., 2009).

**Table 2.** The pH values in examined samples

| Samples | pH values |
|---|---|
| Plain yogurt | $4.51 \pm 0.0^{5\,a}$ |
| Fruit yogurt | $4.43 \pm 0.0^{4\,a}$ |
| Plain rayeb | $4.21 \pm 0.0^{3\,b}$ |
| Fruit rayeb | $4.16 \pm 0.0^{2\,b}$ |

Data were expressed as mean ± 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 highernumber 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 ($5.5\times10^4\pm3.94\times10^3$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 $8.82\times10^4 \pm 0.81\times10^4$ and $5.4\times10 \pm 1.04\times10$ 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 $5.6\times10^4\pm3.68\times10^3$ and $5.02\times10^2\pm0.57\times10^2$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 $1.65\times10^3\pm2.69\times10^2$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 | S. aureus | | Coliforms | |
|---|---|---|---|---|
| | NO(%) | Count | NO(%) | Count |
| Plain yogurt | 2(6.67) | $9.0\times10^2\pm 1.0\times10^{2a}$ | 30(100) | $8.82\times10^4 \pm0.81\times10^{4\,a}$ |
| Fruit yogurt | 6(20) | $4.33\times10^2 \pm 0.42\times10^{2\,b}$ | 4(13) | $5.4\times10 \pm 1.04\times10^{\,b}$ |
| Plain rayeb | 4(13.33) | $4.0\times10^2 \pm 0.57\times10^{2\,b}$ | 0 | 0 |
| Fruit rayeb | 2(6.67) | $10.1\times 102\pm 1.1\times 10^{2\,a}$ | 0 | 0 |

Data were expressed as mean ± 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 yogurtwere serotyped into O111: H2(EHEC) at an incidence rate of 100%.$O_{26}$: $H_{11}$ 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 | | |
|---|---|---|---|---|---|
| | No | % | No | % | |
| E.coli | 22 | 73.3 | 2 | 6.7 | Strains characterization |
| | Serotypes of isolated E.coli | | | | |
| 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 $3.92\times10^4\pm0.81\times10^4$,$2.90\times10^4\pm 1.88\times10^4$, $2.30\times10^2 \pm 0.73\times10^2$, and $2.75\times10^3 \pm 1.42\times10^3$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 $4.2\times10^4 \pm 0.8\times10^4$cfu/gin yoghurt was reported by AbdEllatif et al., (2016). A higher prevalence of 100% with a count of $8.37\times10^5\pm0.96\times10^5$cfu/g was recorded by Osman, (2015). Lower number (40%) but with similar count ($5.6\times10^4\pm1.6\times10^4$cfu/g) was detected in plain yoghurt (Barakat, 2019). Molds were detected at incidence ratesof 66.67, 80, 100, and 100% with counts of $1.74\times10^3 \pm 0.52\times10^3$,$4.25\times10^2 \pm 0.67\times10^2$, $1.95\times10^3 \pm 0.38\times10^3$, and $8.06\times10^2 \pm 0.82\times10^2$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 $4.49x10^5 \pm 0.51 x10^5$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 $1.34\times10^7\pm 0.23\times10^7$cfu/g. Lower mold count (28%) with a level of $1.5 \times10^4\pm0.44\times10^4$cfu/g was detected by Barakat, (2019).Amer, (2017) reported that 44 % of examined

yoghurt samples were contaminated with molds with a count of $3.92\times10^3 \pm 7.67\times 10^2$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 toability 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 form 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 products12 (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) | $3.92\times10^4 \pm 0.81\times10^{4\,a}$ | 20(66.67) | $1.74\times10^3 \pm 0.52\times10^{3\,a}$ |
| Fruit yogurt | 8(26.67) | $2.90\times10^4 \pm 1.88\times10^{4\,b}$ | 24(80) | $4.25\times10^2 \pm 0.67\times10^{2b}$ |
| Plain rayeb | 10(33.33) | $2.30\times10^2 \pm 0.73\times10^{2\,c}$ | 30(100) | $1.95\times10^3 \pm 0.38\times10^{3\,a}$ |
| Fruit rayeb | 8(26.67) | $2.75\times10^3 \pm 1.42\times10^{3\,bc}$ | 30(100) | $8.06\times10^2 \pm 0.83\times10^{2\,a}$ |

Data were expressed as mean ± SEM. Means carrying different superscript letterσ 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 |
| Cladosporum sp. | 2 | 4.3 | 0 | 0 | 12 | 10.9 | 2 | 3.3 |
| Total No. | 46 | 100 | 58 | 100 | 110 | 100 | 60 | 100 |

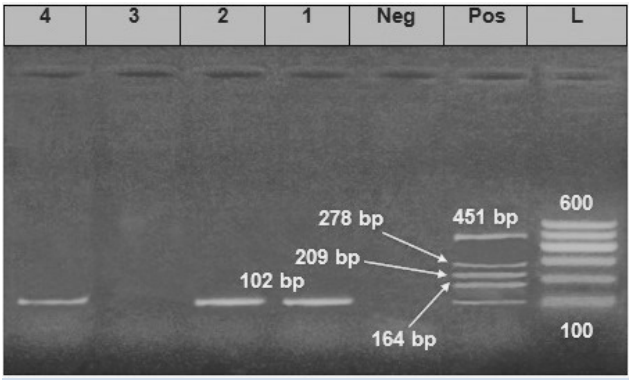


**Fig.1.** Agarose gel electrophoresis of multiplex PCR of *sea* (102 bp), *seb* (164 bp), *sec* (451 bp), *sed* (278 bp), and *see* (209 bp) enterotoxin genes for characterization of *S. aureus*. L: 100 bp DNA ladder; Pos: control positive for *sea*, *seb*, *sec*, *sed*, and *see* genes, Neg: Control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

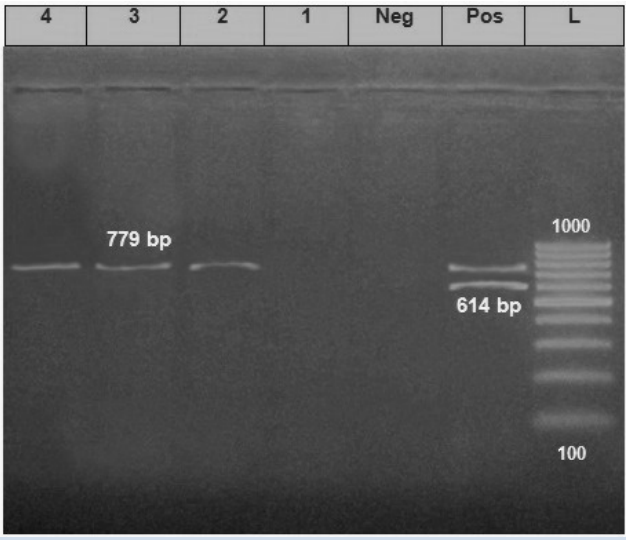


**Fig.2.** Agarose gel electrophoresis of multiplex PCR of *stx1* (614 bp) and *stx2* (779 bp) genes for characterization of *E. coli.* L: 100 bp DNA ladder; Pos: control positive for *stx1* and *stx2*genes, Neg: Control negative, Lanes 2, 3 and 4: Positive for *stx2*, lane 1: negative for *stx2*

## 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.

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